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Factors associated with infectious bursal disease vaccination failure in chickens in Tanzania

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FACTORS ASSOCIATED WITH INFECTIOUS BURSAL DISEASE VACCINATION FAILURE IN CHICKENS IN TANZANIA

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution Sciences and Technology

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ABSTRACT

Infectious bursal disease (IBD) is a viral infection that affects young chicks. The IBD outbreaks in vaccinated chickens are frequently reported in Tanzania. The current study was conducted to determine the possible causes of vaccination failure focusing on knowledge, attitude and practices (KAPs) of vaccine sellers and poultry farmers, maternally derived antibodies (MDA), immunogenic potential of Virgo 7 vaccine and the phylogenetic relationship between the vaccine and the field strains. A cross-sectional study was performed to gather information on KAPs from 384 farmers and 20 veterinary outlets in Dar es Salaam. Results revealed inadequate knowledge of farmers in IBD management and breaches in the cold chain maintenance by vaccine sellers. A total of 60 chicks were experimentally vaccinated with Virgo 7 strain vaccine and titers of induced antibodies assessed. The vaccine induced adequate antibodies against IBDV, this confirming its immunogenic efficacy. Isolated nucleic acids from the vaccine and field strains were sequenced and result shows that field isolates are genetically different from the vaccine strains used in the country. The field isolates belong to the vvIBDV African types, while the vaccines belong to the vvIBDV European or classical virulent types. Putting together results from this study reveals multiple possible reasons which may contribute to vaccine failures. These include poor vaccine handling by farmers and vaccine seller and the genetic disparity between the field and vaccine strains. It is therefore recommended that veterinary regulatory authorities should ensure good vaccine handling practices and considering local virus isolates during vaccine development.

DECLARATION

I, Rukia Saidi do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this submission is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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Rukia Saidi (Candidate name)

14/8/2020

Date

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Dr. Gabriel Shirima (Supervisor 1)

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Prof. Joram Buza (Supervisor 2)

14/08/2020

Date

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of a dissertation entitled "Factors Associated with Infectious Bursal Disease Vaccination Failure in Chicken in Tanzania", in fulfillment of the requirements for the Degree of Master's in Life Science at Nelson Mandela African Institution of Science and Technology (NM-AIST).

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I say God bless you all.

DEDICATION

I dedicate this dissertation to my beloved parents Asiatu Msango and Ally Jumanne, you have always loved me, prayed for me, encouraged and supported me throughout the course of the study.

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LIST OF ABBREVIATION AND SYMBOLS

%	Percentage
µL	Microliter
AGPT	Agar Gel Precipitation Test
ANOVA	Analysis of Variance
BALT	Bronchial-Associated Lymphoid Tissue
BLAST	Basic Local Alignment Search Tool
CALT	Conjunctiva-Associated Lymphoid Tissue
Cdna	Complementary Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetraacetic Acid
EID	Embryo Infectious Dose
ELISA	Enzyme-Linked Immunosorbent Assay
GALT	Gut-Associated Lymphoid Tissue
GDP	Gross Domestic Product
IBD	Infectious Bursal Disease
IBDV	Infectious Bursal Disease Virus
Igg	Immunoglobulin G
Igm	Immunoglobulin M
Kaps	Knowledge, Attitude and Practices
Km	Kilometer
KNCHREC	Northern Zone Health Research Ethics Committee
MDA	Maternally Derived Antibody
MEGA	Molecular Evolutionary Genetics Analysis
mL	Milliliter
NCBI	National Center for Biotechnology Information
OD	Optical Density
RNA	Ribonucleic Acid
Rpm	Revolution per Minutes
RT PCR	Real-Time Polymerase Chain Reaction
RT	Reverse Transcriptase
S/P	Sample to Positive Ratio
SPSS	Statistical Programme for Social Science

Sq	Square
VP	Viral Protein
Vvibdv	Very Virulent Infectious Bursal Disease Virus

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

The livestock industry is one of Tanzania's agricultural sub-sectors contributing about 6.9% of the country's gross domestic product as reported by National bureau of statistics [NBS] (2017). The livestock sector contributes to the national economy in terms of production of raw materials for industry, food production and creation of employment (Silva *et al.*, 2017). Poultry as one of the classes of livestock is an emerging farming industry, which provides animal protein to the urban and peri-urban populations, source of income and creates employment opportunities (Pauw & Thurlow, 2010). However, the poultry industry is challenged by a lack of quality feeds, poor growth rate, prevalent diseases and poor sanitation. Major diseases that impede the development of poultry industry are newcastle disease, fowl pox, infectious coryza, infectious bursal disease (IBD), parasitism and nutritional related deficiencies (Musa *et al.*, 2010). Vaccination is regarded to be the effective way to prevent most of these diseases, particularly infectious bursal disease, newcastle disease, infectious coryza and fowl pox that cause high morbidities and mortalities. Infectious bursal disease has been a great challenge to the poultry industry world-wide for a long time. However, the disease observed to be more challenging particularly for the past two decades following emergence of new pathotypes such as variant and very virulent strains (Sainsbury, 2000). The IBD is one among the most important immunosuppressive diseases that creates serious economic problems for the poultry industry worldwide (Mutinda, 2016). The economic impacts of IBDV are diverse that include not only the direct losses due to morbidity and mortality, but also immunosuppression caused by this virus exacerbating infections with other pathogens (Kurukulasuriya, 2017). Thus, IBDV is one of the most important viral pathogens of commercial poultry.

Infectious bursal disease is an acute and extremely contagious viral disease which affects growing chickens between the age of three to six weeks (Swai *et al.*, 2011). The IBD is caused by infectious bursal disease Virus (IBDV). The disease is also named as "Gumboro disease", because the first case occurred in Gumboro, Delaware, USA, (Cosgrove, 1962).

The IBD virus belongs to the family *Birnaviridae* under the genus *Avibirnavirus* (Brown, 1986). It is non-enveloped and double-stranded RNA virus (Eterradossi *et al*., 2008). There are two IBDV serotypes reported worldwide namely, serotype I and II. However, it was reported that only serotype I contains pathogenic strain to chickens. The serotype I is further categorized as very virulent (VV), mild, intermediate, classical virulent and antigenic variants (Ching *et al.*, 2007). Serotype II viruses are non-pathogenic to chickens, but mostly isolated from other birds like turkey and guinea fowl without manifesting clinical signs (Ismail *et al.*, 1988).

The IBDV contains segment A and B which have been enclosed within non-enveloped icosahedral capsid. The segment A encodes viral proteins 2 (VP2), viral proteins 3 (VP3), viral proteins 4 (VP4) and viral proteins 5 (VP5) (Lejal *et al.*, 2000). The VP2 contains essential neutralizing antigenic areas and produces a protective immune response. Furthermore, the majority of the amino acids changes between antigenically different IBDVs are occurred in the hyper-variable region of VP2 (Fahey *et al.*, 1989). That is why VP2 is used for the IBDV identification and strain variation studies. The VP4 is a virus-encoded serine protease which is responsible for the cleavage of the polyprotein (Birghan *et al.*, 2000). Viral protein 3 is dimeric in structure and it is considered to be a group-specific antigen because it is recognized by monoclonal antibodies directed against VP3 from strains of both serotype 1 and 2 (Becht *et al.*, 1988). It is a multifunctional protein involved in determining the morphogenesis of the virus particle (Maraver *et al.*, 2003). The nonstructural protein VP5 is involved in virus release and apoptosis (Liu & Vakharia, 2006). Segment B contains VP1, which is responsible for viral genome replication and mRNA synthesis (Jackwood *et al.*, 1982).

The main clinical signs manifested by diseased chickens are watery diarrhoea, immunosuppression hemorrhagic syndrome, depression, dehydration, inappetence, ruffled feathers, vent picking and reluctant to rise (OIE, 2000). The IBD virus affects B cells in immature stage and therefore induces immunosuppression (Meulemans, 2000). Since the chickens become immunosuppressed, they also develop a weak immune response when vaccinated against other diseases (Müller *et al.*, 2012). Therefore, infectious bursal disease is considered as one of the setbacks in poultry farming around the world (Eterradossi *et al.*, 2008).

The prevention of this disease is mainly through vaccination and strictly bio-security measures (Müller *et al.*, 2012). Adequate bio-security measures include restriction of farm visit and avoidance of mixing chickens from different flocks. This is very useful for disease prevention, especially before disease outbreak (Nespeca *et al.*, 1997).

There are two types of IBDV vaccines namely; live attenuated and inactivated vaccines. Proper timing for administration of live vaccines is extremely important, since when the vaccine is administered in early age the circulating maternally derived antibodies (MDA) tend to interfere with vaccine virus hence affect its performance (Müller *et al.*, 2012). Inactivated vaccines observed to be very effective in vaccinating parent stocks before laying so that the protective antibodies can be transferred to their progenies.

Maternally derived antibodies (MDA) are pathogen specific antibodies which vertically transferred from hen to the hatching progeny through the egg yolk (Cardenas-Garcia *et al.*, 2019). The MDA are absorbed from the yolk into the blood circulation of the chick and may work similarly to naturally induced antibody (Faulkner *et al.*, 2013). The MDA can prevent clinical disease by passive vaccination for various pathogens in the chick (Faulkner *et al.*, 2013). Maternal derived antibodies against specific disease play an important role in protection of chicks against that disease before the development of active immunity (Ahmed & Akhter, 2003; Heller *et al.*, 1990). Studies show that the level of MDA decrease with time and it reach a point where it become below the protective amount hence the chicks can be susceptible to the infection (Faulkner *et al.*, 2013). On the other hand, chicks vaccinated while having high level of MDA against infectious bursal disease virus resulting in vaccine failure, due to of neutralization of the vaccine virus (Al-Natour *et al.*, 2004; Naqi *et al.*, 1983). For that reason, it is very important to consider the half life of MDA in designing vaccination programs for chickens to minimize the cross reaction between circulating maternal antibodies and the vaccines (Gharaibeh & Mahmoud, 2013).

For a long time, IBD has been reported to be one of the major hindrance factor for the poultry farming globally (Dey *et al.*, 2019; Sainsbury, 2000). However, the disease has become more devastating especially in the last two decades after the appearance of very virulent and variant strains (Mutinda, 2016). Tanzania has also reported the existence of African and European vvIBDV strains (Kasanga *et al.*, 2007). These strains affect both productivity and profitability in the poultry industries of both industrialized and developing countries (Sainsbury, 2000).

Before 1980, IBDV strains were of low virulence and mortality and were effectively controlled through vaccination (Berg, 2000). However, IBD vaccine failures have been reported worldwide at the mid of 1980s (Berg, 2000).

Vaccination failure could occur due to the fact that the local strains available in the fields are genetically different from vaccine strains (Chawinga, 2016), inappropriate administration of vaccines (Rosenberger *et al.*, 1987), lack of vaccine potency (Ismail & Saif, 1991), interaction with maternally derived antibody (MDA) especially when vaccine is administered at early age, farmers' inadequate knowledge about the disease and non-compliance with the vaccine's cold chain specifications (Mbuko *et al.*, 2010).

The first report of infectious bursal disease in Tanzania came from Dar es Salaam and the Coast regions in 1988 (Kapaga *et al.*, 1989). After that, the disease spread all over the country in poultry farming units that led to significant economic losses to farmers (Kasanga *et al.*, 2007). Even though chickens have been vaccinated against IBD, some farmers still reporting IBD outbreaks in both vaccinated and non-vaccinated chickens. This indicates an inefficiency or failure of vaccine used in the country (Kasanga *et al.*, 2007). No evidence of research carried out to investigate the possible causes of vaccine failures, even though farmers reported IBD outbreak in vaccinated flocks. Therefore, the current study was conducted to explore the possible causes of vaccine failure including characterization and comparison of field IBDV strains and vaccine strains, assessment of immunogenic potential of the Virgo 7 IBD vaccine commonly used in chicks in Tanzania and the knowledge, attitude and practices (KAPs) of poultry farmers towards managing IBD vaccines.

1.2 Statement of the Problem

Infectious bursal disease is among the most devastating disease in the poultry farming because of mortality and exposing chickens to be more susceptible to secondary infections (Kurukulasuriya, 2017). The disease is observed to be more severe in developing countries due to poor bio-security practices such as poor hygiene (Mohamed *et al.*, 2014).

The farmers in Tanzania use different types of IBDV vaccines and vaccination programs. However, IBD outbreaks are still reported in both vaccinated and non-vaccinated chickens (Kasanga *et al.*, 2007). No evidence of research carried out to investigate the possible causes of vaccine failures even though farmers reported IBD outbreak in vaccinated and non-

vaccinated chicken flocks. Therefore, the current study aimed to examine the possible causes of vaccination failure including characterization of field IBDV strains against vaccine strains, assessment of immunogenic potential of the Virgo 7 IBD vaccine commonly used in chicks in Tanzania and the KAPs of poultry farmers towards managing IBD vaccines.

1.3 Rationale of the Study

The farmers in Tanzania use different types of IBDV vaccines and vaccination programs to control IBD. However, IBD outbreaks are still reported in both vaccinated and non-vaccinated chickens. This indicates an inefficiency of vaccines used in the country. Therefore, the current study aimed to examine the possible causes of vaccination failure in order to improve the poultry industry. Poultry farming provides animal protein to the urban and peri-urban populations, source of income and creates employment opportunities.

1.4 Objectives

1.4.1 Main Objective

To assess the associated hindrance factors for the most commonly used infectious bursal disease vaccines for future improvement in poultry productivity in Tanzania.

1.4.2 Specific Objectives

(i) To assess the knowledge, attitude and practices of poultry farmers in managing IBD.

(ii) To examine the decreasing trend of maternally derived antibodies against IBDV.

(iii) To assess the immunogenic potential of the Virgo 7 intermediate hot strain IBD vaccines (VIR-114) in chickens.

(iv) To characterize the phylogenetic relationship between field IBDV strains and currently used IBDV vaccine strains in Tanzania.

1.5 Research Questions

(i) What is the level of KAPs of poultry farmers in management of IBD?

(ii) What is the level of maternally derived antibodies against IBDV?

(iii) What is the level of antibody titer produced by (Virgo 7 intermediate hot strain IBD vaccines (VIR-114) in chickens?

(iv) What is the phylogenetic relationship between field and imported IBD vaccines available in Tanzania?

1.6 Significance of the Study

This study pointed out the antigenic differences between circulating field IBDV strains and the current IBDV vaccine strains used in Tanzania. The study provides the information on the KAPs of poultry farmers and vaccine sellers on vaccine handling in Dar es Salaam region. Understanding of genetic disparity between the field and vaccine strains help in the development of a vaccine which will consider local IBDV isolates. The study findings will provide the baseline data on the KAPs of poultry farmers and vaccine sellers on vaccine handling in other regions within the country.

1.7 Delineation of the Study

The study was limited on investigation of possible causes for vaccination failure. It was based on assessing knowledge, attitude and practices of poultry farmers and vaccine sellers toward IBD vaccines management and immunogenic potential of Virgo 7 vaccine. Furthermore, the study involved assessment of phylogenetic relationship between field IBDV strains and currently used IBDV vaccine strains in Tanzania.

CHAPTER TWO

LITERATURE REVIEW

2.1 Infectious Bursal Disease

Infectious bursal disease (IBD) is an acute and highly contagious viral disease affecting the immune system of poultry in an immature stage (three to six weeks of age). The disease is caused by the infectious bursal disease virus (IBDV). The IBD virus belongs to the family *Birnaviridae* under the genus *Avibirnavirus* (Brown, 1986). It is non-enveloped RNA virus with double strands (Eterradossi *et al.*, 2008). The disease is also termed as "Gumboro disease", because the first case occurred in Gumboro, Delaware, USA (Cosgrove, 1962).

There are two IBDV serotypes reported worldwide which are serotype I and II. However, it was reported that only serotype I contain pathogenic strain to chickens. The serotype I is further categorized as very virulent (VV), mild, intermediate, classical virulent and antigenic variants (Ching *et al.*, 2007). Serotype II viruses are non-pathogenic to chickens, but mostly isolated from other birds like turkey and guinea fowl without manifesting clinical signs (Ismail *et al.*, 1988).

The IBDV contains segment A and B which have been enclosed within non-enveloped icosahedral capsid. The segment A encodes viral proteins 2 (VP2), viral proteins 3 (VP3), viral proteins 4 (VP4) and viral proteins 5 (VP5) (Lejal *et al.*, 2000). The VP2 contains essential neutralizing antigenic areas and produces a protective immune response. Also the majority of the amino acids changes between antigenically different IBDVs are occurred in the hyper-variable region of VP2 (Fahey *et al.*, 1989). That is why VP2 is used for the IBDV identification and strain variation studies. Viral protein 4 is a virus-encoded serine protease which is responsible for the cleavage of the polyprotein (Birghan *et al.*, 2000). Viral protein 3 is dimeric in structure and is considered to be a group-specific antigen because it is recognized by monoclonal antibodies directed against VP3 from strains of both serotype 1 and 2 (Becht *et al.*, 1988). It is a multifunctional protein involved in determining the morphogenesis and act as scaffolding element during the assembly of the virus particle (Maraver *et al.*, 2003). The non-structural protein VP5 is involved in virus release and apoptosis (Liu & Vakharia, 2006). Segment B contains VP1 which is responsible for viral genome replication and mRNA synthesis (Jackwood *et al.*, 1982).

The infectious bursal disease is very devastating because of increased vulnerability of infected chickens to other diseases and affect vaccine efficiency (Saif, 1991). The disease is mainly targeting the immune system of the chickens (B lymphocyte). That is why it end up causing massive destruction of B cells in lymphoid organ and immunosuppression that increase disease susceptibility and secondary infection of infected birds such as *Escherchia coli*, salmonella and mycoplasma (Meulemans, 2000).

Infectious bursal disease has become more devastating especially in the last two decades following the detection of very virulent and variant IBDV strains (Mutinda, 2016). Tanzania also has reported the existence of African and European vvIBDV strains (Kasanga *et al.*, 2007). These strains affect both productivity and profitability in the poultry industries all over the world (Sainsbury, 2000). Before 1980, IBDV strains were less virulent with low mortality and were effectively prevented through vaccination (Berg, 2000). However, IBD vaccine failures have been reported worldwide at the end of 1980s (Berg, 2000). Vaccination failure could occur due to the fact that the local strains available in the fields are genetically different from vaccine strains (Chawinga, 2016), inappropriate vaccine preparation and administration (Rosenberger *et al.*, 1987), strength of vaccine virus (Ismail & Saif, 1991), interaction with maternally derived antibody (MDA) especially when vaccine is administered at early age and pathogenicity of circulating IBDV strains (Sainsbury, 2000). Farmers' inadequate knowledge about the disease and non-compliance with the vaccine's cold chain specifications might also cause failure of vaccination process (Mbuko *et al.*, 2010).

The first report of infectious bursal disease in Tanzania came from Dar es Salaam and the coast regions in 1988 (Kapaga *et al.*, 1989). After that, the IBD spread all over the country in poultry farming units that led to significant economic losses to farmers. Even though chickens have been vaccinated against IBD, some farmers still reporting IBD outbreaks in both vaccinated and non-vaccinated chickens. This indicates an inefficiency of vaccines used in the country (Kasanga *et al.*, 2007).

2.2 Epidemiology

2.2.1 Host Range

Chickens are the only bird among avian species which develop IBD after being infected by serotype 1 viruses. Pekin ducks were reported to be asymptomatic carrier for serotype 1

viruses (McFerran *et al.*, 1980), turkeys for serotype 2 (Ismail *et al.*, 1988), guinea-fowl, phasianus colchicus and ostriches for serotype 2 viruses (Guittet *et al.*, 1982). In addition, neutralizing IBD antibodies have been found in different types of undomesticated duck, goose, tern, puffin and penguin. This suggesting that wild birds may possibly play part in the epidemiology of IBD (Wilcox *et al.*, 1983).

2.2.2 Transmission and Pathogenesis

The infectious bursal disease is an acute and extremely contagious viral disease that is transmitted primarily through the faecal-oral route as the virus is shed in the faeces. The incubation period for IBD varies between 2 to 4 days and the diseased chickens begin to shed the virus 24 hours after infection (Lawal *et al.*, 2014). The IBD virus can live within the environment at a pH range of 2 to 12, in poultry houses for 122 days, in feed and water for 52 days (Müller *et al.*, 2012). The disease can be transmitted directly between bird to bird and indirectly through contaminated feeds, water, faeces, equipment, people's clothing and shoes (Benton *et al.*, 1967). Infectious bursal disease virus is very resistant to harsh condition and can survive for months in the poultry house environment. Elimination of the virus can be extremely difficult since the virus is resistant to several disinfectants (Benton *et al.*, 1967).

Following ingestion of the IBDV, phagocytic cells such as macrophages transferred it from the gut to the other tissues. The virus then reaches the bursal of fabricious via blood to destroy lymphoid follicles including B lymphocytes in the secondary lymphoid tissues such as GALT (gut-associated lymphoid tissue), CALT (conjunctiva), BALT (bronchial) and caecal tonsil (OIE, 2000). Necrosis and renal failure are the main cause of death for this disease (Muskett *et al.*, 1985).

Cells which produce IgM are the one which is targeted by the virus. Therefore, in the acute phase of the disease, the circulating IgM are decreased (Rodenberg *et al.*, 1994). However circulating IgG level remains the same (Kim *et al.*, 1999). The bursal is atrophied once the bursal follicles become depleted of B lymphocytes. Severe damage of lymphoid cells occurred following replication of viruses. Apoptosis of the neighbouring non-infected B cells causes destruction of the bursa morphology. At this period, a significant quantity of viral load can be found in other lymphoid organs such as caecal, tonsils and spleen (Tanimura & Sharma, 1997). The destruction of lymphocyte populations causes immunosuppression and hinder immunological maturation of infected birds (Sharma *et al.*, 2000).

2.3 Clinical Presentation

Clinical presentation of IBD depends on the pathogenicity of the IBDV strains, age and breed of affected chickens (Mutinda, 2016). The most important clinical signs are in appetence, white watery diarrhoea, ruffling of feathers, vent picking, trembling, prostration and inability to move (Mohabe, 2012). The IBD can be presented in form of hyperacute, classical and immuno-suppressive.

The classical virulent IBDV strains are the ones which cause classical form of IBD. The disease is presented by acute depression followed by typical signs and damages. The classical form of disease results in mortality of approximately 10 to 50% (OIE, 2000).

The hyper-acute form is caused by very virulent IBDV strains. The affected chickens show severe clinical signs which lead to high mortality rates. The hyper-acute form of disease cause mortality of approximately 50 to 84% with typical signs and damage (Mutinda, 2016). Other studies reported that mortality caused by very virulent IBDV could vary between 40 to 84% in specific pathogen free (SPF) chickens, 60% in layers, and 30% in broilers (Jackwood *et al.*, 2009).

The immunosuppressive form is mainly reported in United States. This form of disease is caused by IBDV strains with low pathogenicity and variant strains, such as the Delaware variant strains, which partially resistant to neutralization by circulating antibodies (Snyder, 1990). Variant IBDVs induce little clinical signs and no mortality (Mohabe, 2012).

2.4 Diagnosis

Preliminary diagnosis of IBD involves consideration of flock's history, clinical signs and findings at necropsy. Differential diagnoses with regard to clinical signs, include newcastle disease, mycotoxicosis, infectious bronchitis and inclusion body hepatitis. The disease may also be confused with chicken anaemia and Marek's in subclinical and immunosuppressive forms (Eterradossi *et al.*, 2004). Nevertheles, these can easily be differentiated at necropsy. Confirmation of the disease can be done by detecting and characterizing specific IBD virus. For specific detection of IBD virus in affected tissues immunofluorescence tests, in-situ hybridization and based on labeled complementary cDNA sequence probe, are commonly used. Furthermore, viral RNA detection in infected chicken can be done by RT-PCR (Jackwood *et al.*, 1996). Serology is also used in disease diagnosis by detecting IBD

antibodies, this is done by using agar gel precipitation test (AGPT) and ELISA (Angani *et al.*, 2014). Furthermore, serological techniques are frequently used to identify the immunologic response in disease outbreak and evaluation of vaccine performance.

2.4.1 Gross Lesions

The extent of the lesions depends on the virulence of the IBD virus strains (Mutinda, 2016). Chickens which die from the acute condition exhibit dehydration of the subcutaneous fascia, inguinal, pectoral and thigh muscles (Cosgrove, 1962; Hirai *et al.*, 1981). Haemorrhages appear in the pectoral and thigh muscles and occasionally appear on the mucosa at the proventriculus junction and plicae of the bursa (Huff *et al.*, 2001). Kidneys enlarge and become pallor with an accumulation of urate in tubules (Cosgrove, 1962). Spleen observed to be enlarged, with tiny grey foci evenly distributed all over the parenchyma (Hirai *et al.*, 1981).

Infection with classic viruses is demonstrated by increasing of the bursa dimension accompanied by inflammation. Once the inflammation subsides, the bursal is rapidly atrophied. Chickens that die early observed to double the size of the bursa because of oedema. The colour of the bursa changes to light yellow with striations. The bursa resume to its normal weight at day five. However, it continues to decrease in size from day eight onward and becomes one-third of its original weight (Hirai *et al.*, 1981). The vvIBDV strains cause a massive reduction in thymic weight and massive damages in bone marrow, thymus, cecal tonsils and spleen. Nevertheless, the bursal damages are similar to other subtypes (Hasan *et al.*, 2010).

2.4.2 Histological Lesions

Infectious bursal disease virus causes severe damage to lymphocytes hence causes damage of the bursa of fabricius and other lymphoid organs (Mahgoub, 2012). The death of B lymphocytes is easily diagnosed at day one of infection (Mahgoub, 2012). At day three of infection, an inflammatory reaction characterized by heterophils infiltration, oedema, congestion and haemorrhages appeared in the organ. At day four, the acute inflammatory responses decrease since necrotic materials have been cleared by phagocytic cells like macrophages. The highly pathogenic strains of IBDV causes severe thymocyte damage when compared to less virulent strains (Tanimura *et al.*, 1995). Acute heterophilic inflammation

and damage of lymphocytes have been observed in the caecal tonsils on day five following infection (Helmboldt & Garner, 1964). Damage of the liver tissues and fatty deterioration of hepatocytes have been reported in IBDV infection (Ma *et al.*, 2013).

2.5 Prevention and Control

The prevention of this disease is mainly through vaccination and strictly bio-security measures (Müller *et al.*, 2012). There are two main types of IBDV vaccines namely; live attenuated vaccine and inactivated vaccines. Proper timing for administration of live vaccines is extremely important because when the vaccine is administered in early age the circulating MDA tend to interfere with vaccine virus hence affect its performance (Müller *et al.*, 2012). Inactivated vaccine is known to be very effective in vaccinating parent stocks before laying so that the protective antibodies can be transferred to their progenies.

Furthermore, adequate bio-security measures including restriction of farm visit and avoidance of mixing chickens from different flocks are very useful for disease prevention, especially before disease outbreak. Implementation of bio-security measures after the disease outbreak will be useless since the virus can live in the chicken house and environment for a long period of time.

2.5.1 Infectious Bursal Disease Vaccine

(i) Live Attenuated Vaccines

This vaccine is developed by reducing the virulence of a pathogen, but still keeping its viability. The vaccines are mainly categorized into mild, intermediate and intermediate plus (hot) depending on the extent of attenuation (Rautenschlein *et al.*, 2005).

Mild vaccines are highly attenuated, breaking through very low levels of MDA. Therefore, they are no longer applicable in the commercial environment. The mild vaccine reported to cause limited bursal damage.

Intermediate and intermediate plus (Hot) vaccines induce severe bursal lesions and could easily revert back to virulence resulting in disease and loss of production (Hair-Bejo *et al.*, 2004). Intermediate vaccine has few side effects, but limited efficacy against vvIBD in the field and in situations of high infectious pressure (Haddad *et al.*, 1997). Hot vaccines have a

powerful and aggressive action, but can damage the bursa, impairing the immune response and response to other vaccinations. Proper timing is critical in the administration of live vaccines to chicks due to interference with MDA (Müller *et al.*, 2012). However, high parental immunity is the most important in protecting chicks from IBDV infection during the early age of life while the bursa is more prone to IBDV infection (Hitchner, 1976).

Administration of this vaccine is generally through drinking water or spraying. When MDA is not detected in chickens, the mild vaccine is normally given at day one of age. If MDA is detected in early age, the vaccine is administered after the decline of MDA. However, the appropriate vaccination time is established by conducting serological test so as to establish the level of MDA. Currently, formulation of vaccines has been advanced in such a way that can be administered at day eighteen in hatchery through in-ovo route. Most of the live vaccines used in chicks are suitable for mass vaccinations that do not require an adjuvant and can replicate in the bird to stimulate both humoral and cell-mediated immunity (Müller *et al.*, 2012).

(ii) Inactivated Vaccine

This vaccine consists of virus particle that has been grown in culture and then killed. The vaccine does not replicate in the bird. It is costly to produce and administer but, is very useful in parent flocks prior to laying to provide passive immunity to offspring via MDA (Haddad *et al.*, 1997). The inactivated vaccines must have an antigenic content that is high enough to induce high immunity in parent flocks that can be passed to progeny at protective levels (Rosenberger *et al.*, 1987). Infectious bursal disease vaccines have not been very successful in different parts of the world due to progressive changes in antigenicity and poor handling of the vaccines (Müller *et al.*, 2012; Mutinda *et al.*, 2014). However, vaccination still remains the best option in controlling IBDV in the field besides bio-security.

2.6 Vaccination Failure

Vaccination failure is described as the inability of the vaccinated chickens to produce sufficient amount of antibodies following administration of the vaccine (Abdullahi *et al.*, 2009). Vaccination failure could occur due to the fact that the local strains available in the fields are genetically different from vaccine strains, inappropriate vaccine preparation and administration, interaction with maternally derived antibody (MDA) especially when vaccine

is administered at early age, pathogenicity of circulating IBDV strains and non-compliance with the vaccine's cold chain specifications.

2.6.1 Non-usage of Local Antigen

There are several serotypes of infectious bursal disease virus; these serotypes could be common in one region and different from other region. Usage of vaccine which contains IBD strains which are genetically different from field isolates may offer low protection or might not protect the chicks completely, hence result to vaccine failure (Mutinda *et al.*, 2014).

2.6.2 Presence of Maternally Derived Antibodies

Establishment of the level of maternally derived antibody (MDA) before vaccination is extremely important. The high level of MDA's provides protection at early age when the chicks are more likely to be affected by IBDV. However, high MDA titer may interfere with vaccine through neutralization (Sarachai *et al.*, 2010) and thus recommended to vaccinate when the MDA titers waned out below protection level.

2.6.3 Non-adherence to the Recommended Storage Temperature

Once the vaccine has been formulated, the storage is very critical. The vaccines must be stored according to manufacturer recommendation. Live vaccines are always stored at 4 – 8 °C. Most of the vaccines used in chickens are heat intolerant. For this reason, adherence to the recommended storage temperature is crucial for best performance of vaccines. The cold chain breaks due to power cutoff, nonfunctional storage equipment, fluctuation of temperature during transportation and lack of standby generator are the commonly problems encountered in vaccine handling especially in developing countries (Hanjeet *et al.*, 1996; Simba & Msamanga, 1994; Sudarshan *et al.*, 1994; Thakker & Woods, 1992).

2.6.4 Non-compliance to Vaccination Protocol

Poor sanitation, lack of adherence to vaccination regimen, use of chlorinated water in vaccine reconstitution, may compromise the vaccine efficacy (Rosenberger *et al.*, 1987). Furthermore, farmers' inadequate knowledge about the disease and non-compliance with the vaccine's cold chain specifications may also lead to vaccination failure (Mbuko *et al.*, 2010).

2.7 Maternally Derived Antibodies

Maternally derived antibodies (MDAs) are pathogen specific antibodies which vertically transferred from hen to the hatching progeny through the egg yolk (Cardenas-Garcia *et al.*, 2019). Maternally derived antibodies are absorbed from the yolk into the blood circulation of the chick and may work similarly to naturally induced antibody (Faulkner *et al.*, 2013). The MDA can prevent clinical disease by passive vaccination for various pathogens in the chick and the MDA have a characteristic half-life similar to host antibodies before they naturally degrade in the chick (Faulkner *et al.*, 2013). It is thought that MDAs bind to the target antigen preventing correct antigen presentation to B cells and initiation of a primary immune response.

Maternal derived antibodies against specific disease play an important role in protection of chicks against that disease before the development of active immunity (Ahmed & Akhter, 2003; Heller *et al.*, 1990). Studies show that the level of MDA decrease with time and it reach a point where it become below the protective amount hence the chicks can be susceptible to the infection (Faulkner *et al.*, 2013). On the other hand, chicks vaccinated while having high level of MDA against infectious bursal disease virus resulting in vaccine failure, due to neutralization of the vaccine virus (Al-Natour *et al.*, 2004; Naqi *et al.*, 1983). For that reason, it is very important to consider the half-life of MDA in designing vaccination programs for chickens to minimize the cross reaction between circulating maternal antibodies and the vaccines (Gharaibeh & Mahmoud, 2013). Several studies have been conducted with regard to duration of MDA. The study done by Skeeles (1979) and Wood (1981) showed that the half-life of MDA against IBDV varied between three to eight days.

2.8 Enzyme Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a labeled immunoassay which is considered as the gold standard of immunoassays (Lequin, 2005). This immunological test is very sensitive and is used to detect and quantify substances such as antibodies, antigens, proteins, glycoproteins and hormones (Aydin, 2015). The detection of these products is accomplished by the complexing of antibodies and antigens to produce a measurable result. An antibody is a type of protein produced by an individual's immune system. This protein type has specific regions that bind to antigens. When antigen bound to an antibody, it induces a cascade of

events through the body's immune system (Shah & Maghsoudlou, 2016). This interaction is utilized in ELISA test and allows for the identification of specific protein antibodies and antigens with only small amounts of a test sample. The ELISA test is used in the diagnosis of IBDV, newcastle disease and Brucellosis, among others. Antigen capture ELISA (Ac-ELISA) had been used for the detection of IBDV antigens directly from infected tissues (Snyder *et al*., 1988).

The ELISA test has been categorized into direct, indirect, sandwich, and competitive type. Enzyme-linked immunosorbent assays are performed in polystyrene plates, typically in 96 well plates that are coated to bind protein very strongly. Depending on the ELISA type, testing requires a primary and/or secondary detection antibody, antigen, coating antibody/antigen, buffer, wash and substrate (Tiscione, 2018). The primary detection antibody is a specific antibody that only binds to the protein of interest, while a secondary detection antibody is a second enzyme-conjugated antibody that binds a primary antibody that is not enzyme-conjugated (Aydin, 2015). Generally, ELISA test involves coating (with either antigen or antibody), blocking, detection and final read.

Detection is carried out by the addition of a substrate that can generate a color. There are many substrates available for use in ELISA detection. However, the most commonly used is horseradish peroxidase (HRP) and alkaline phosphatase (ALP). The substrate for HRP is hydrogen peroxide and results in a blue color change (Tiscione, 2018). Alkaline phosphatase measures the yellow color of nitrophenol after room temperature incubation periods of 15 to 30 minutes and usually uses P-Nitrophenyl-phosphate (pNPP) as its substrate (Sali, 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical Approval

The permission to carry out the study was obtained from the relevant bodies including Executive Directors of Ilala, Kinondoni, Temeke, Ubungo and Kigamboni municipals. The study was approved by the Northern Zone Health Research Ethics Committee (KNCHREC) and all the participants involved in the study were provided with a pre-informed consent form to express their willingness to participate on the study.

3.2 Study Site and Population

The study was conducted in Dar es Salaam region since it is the largest city in Tanzania. Dar es Salaam is located in the Eastern zone of Tanzania. The region is divided into five municipals namely; Ilala, Kinondoni, Temeke, Ubungo and Kigamboni. It covers 1 393 Km^2 of land. The population census conducted in 2012 shows that Dar es Salaam has a human population of 4 364 541. The region was purposively selected since it is the largest center for commercial chicken production and a major consumer of poultry products in Tanzania (Mubito *et al.*, 2014).

3.3 Study design, Sampling and Sample Size

The cross-sectional study design was employed to collect data which was used to assess knowledge, attitude and practices of selected commercial poultry farmers on IBD. The questionnaire was translated into "Swahili", the national language that is understood by all Tanzanians. The questionnaire was pretested on a few poultry farmers from Ilala municipal to test the sequence of the questions as well as the estimated time for each questionnaire. Small modifications were made after the pilot study. Data from the pilot study was not used in the final analysis.

A two-level multistage sampling technique was employed to obtain poultry farmers. The first stage involved the selection of study wards within five municipals. Wards were obtained by simple random sampling. The second stage involved obtaining commercial poultry farmers from each of the selected wards in stage one. Within a ward, the list of poultry keepers was

used as a sampling frame from which respondents were randomly selected. A total of 384 poultry farmers were randomly selected from different wards. The inclusion criteria were a poultry farmer who had 100 and above chickens. Three wards were randomly selected from each of the five municipals in Dar es Salaam. This made a total of 15 study wards. In each ward, a minimum of 25 poultry farmers were selected.

To get the number of participants, a formula for cross-sectional studies of (Kish & Lisle, 1965) was used.

$$n = Z^2 P\ (1 - P)/e^2$$

Where n = sample size, Z = level of confidence, P = baseline level of selected indicator and e = margin of error.

The values were set at P = 0.5 (50%), since this was the first study to be done on assessment of KAPs of IBD in Tanzania, the maximum level of knowledge was assumed to be 50% , Z = 1.96 (at 95% confidence interval) and e = 0.05.

Purposive sampling technique was employed to obtain the number of veterinary shops included in the study. A total of 20 veterinary shops were purposively selected from different wards. The selected veterinary shops were the one that provided services to these farmers including selling vaccines. Purposive sampling technique was used in order to link the information between vaccine handling in the shop, vaccine handling from the shop to the farm and vaccine reconstitution and administration by the farmers. Therefore, all farmers interviewed in this study were asked to mention the place where they get IBD vaccines and all shops mentioned by farmers were included in the study.

3.4 Data Collection Tools and Method

A semi-structured questionnaire and checklist were used for a collection of data from all selected poultry farmers and vaccine sellers respectively. The personal interview was used to gather information from 384 poultry farmers and 20 veterinary outlets. The interviews focused on vaccination practices, vaccine handling from the shop, vaccine transportation and general IBD management. Data on the occurrence of IBD in vaccinated chickens and source of vaccines used in the country were also asked. Direct observations were also made to

compare with respondent's answers and thus the respondents were cross-questioned to have the actual information.

3.5 Examination of Decreasing Trend of Maternally Derived Antibodies

The experimental study started with preparation of chicken house. The chicken house was secured from Sokoine University of Agriculture (SUA). The selected room for the experiment were properly cleaned, disinfected with Virid® and left for 2 weeks prior introduction of chicks to prevent any possible infections. The brooder house was made by using a clean ceiling board and the size of the brooder house was increased as the age of chick's progress.

Thirty (30) day old commercial broiler chicks were purchased from a poultry hatchery in Coast region and raised at SUA farm. These chicks were obtained from parent stocks who were vaccinated against IBD. Furthermore, chicks were given anticoccidial drug (Amprollium) at day 7 of age for 5 days and antibiotics for 5 days to prevent early infections. Chicks were raised in deep litter house made of rice husks. Electric bulbs with 200 W were installed in the brooder house to keep the room warm with the temperature ranged between 35 to 40 °C. Chicks were wing-tagged for individual identification. All chicks were raised in the environment with similar conditions for the whole period of study. Feed and water were supplied adilibitum. This trial was done as per the Tanzania Animal Welfare Act of 2008 with efforts to minimize stress.

The sample size of 30 chicks was proposed based on the previous study done by Mutinda (2016) with some adjustment. In this study, 20 chicks were proposed, however, in the current study, the number was adjusted to 30 chicks to compensate death if occurred. Blood was collected from wing vein of the chicks at specific intervals up to day 42. The chicken was handled by positioning it horizontally on its back, one hand hold the legs and the wing to support the chicken, the other hand was used to draw the blood. Sterile plastic disposable syringes of 2 mL and 29 gauges were used to collect blood. About 0.5 mL of blood was taken from each chick. The collection of blood started from day 1 up to day 42 (1^{st}, 14^{th}, 21^{st}, 28^{th}, 35^{th} and 42^{nd}). Blood samples were left to clot at room temperature overnight. Each blood sample was centrifuged at 3 000 rpm for 15 minutes to separate and obtain the sera. The

serum was harvested by using pasteur pipette and transferred into eppendorf tubes. All the sera were stored at -20 °C until analysis.

Serological analysis of the study samples was done by ELISA technique using indirect ELISA kits (ID Vet, France) as per manufacturer's instructions. The ELISA kit reagents and serum samples that were stored at 2 – 8 °C and at -20 °C respectively were allowed to thaw at room temperature (25 – 27 °C) prior to analysis. An aliquot (5 µL) of each sample were added to the microtitre plate. Then 245 µL of dilution buffer was added to all wells except to control well A1, B1, C1 and D1. That gave a 1:50 dilution of a serum sample to diluents in the dilution plate. After that, 384 µL of negative control was added to well A1 and B1 and 384 µL of positive control were added to well C1 and D1 of the ID.vet test kit plate. Thereafter, 90 µL of dilution buffer was added to all wells except to control well A1, B1, C1 and D1. Then 10 µL of the pre-diluted samples were added to each corresponding well of the ID.vet test kit plate. This gave 384 µL per well and final dilution of 1:500 in dilution buffer. The plate was covered and incubated for 30 minutes at room temperature. After 30 minutes the plate was emptied and washed three times with 300 µL of wash solution. The plate was then inverted and tapped firmly on absorbent paper. Then 384 µL of the conjugate was added to each well. The plate was covered and incubated for 30 minutes at room temperature. After 30 minutes the plate was emptied and washed three times with 300 µL of wash solution. The plate was then inverted and tapped firmly on absorbent paper to dry. 384 µL of substrate solution was added to each well. The plate was covered and incubated for 15 minutes at room temperature. After that 384 µL of stop solution was added to each well to stop the reaction. The absorbance values were read and recorded at wavelength 450 nm using ELISA reader (Multiscan^{TM} FC Microplate Photometer).

The antibody titer in chicken serum sample was calculated by referring to the positive control. That association was expressed as sample to positive ratio (S/P ratio). To confirm if the test results are valid the mean optical density (OD) value of the positive control should be greater than 0.250 and the ratio of the mean values of the positive and negative controls should be greater than 3.0. Interpretation of the results was done based on the S/P ratio and ELISA antibody titer. The result is positive if the S/P ratio is > 0.3 or antibody titer > 875 and result was considered negative when the S/P ratio is ≤ 0.3 or antibody titer ≤ 875.

3.6 Assessment of the immunogenicity of Virgo 7 strain vaccine

The experimental study started with preparation of chicken house. Chicken house was secured from Sokoine University of Agriculture (SUA). The selected rooms for the experiment were properly cleaned, disinfected with Virid® and left for 2 weeks prior introduction of chicks to prevent any possible infections. The brooder house was made by using a clean ceiling board and the size of the brooder house was increased as the age of chick's progress.

Sixty (60) day old commercial broiler chicks were purchased from a poultry hatchery in Coastal region and raised at SUA farm. These chicks were obtained from parent stocks who were vaccinated against IBD. Furthermore, chicks were given anticoccidial drug (Amprollium) at day 7 of age for 5 days and antibiotics for 5 days to prevent early infections. Chicks were raised in deep litter house made of rice husks. Electric bulbs with 200 W were installed in the brooder house to keep the room warm with the temperature ranged between 35 to 40 °C. Chicks were wing-tagged for individual identification and reared in isolated rooms after 14 days. All chicks were raised in the environment with similar conditions for the whole period of study. Feed and water were supplied adilibitum. This trial was done as per the Tanzania Animal Welfare Act of 2008 with efforts to minimize stress.

The sample size of 60 chicks was proposed based on the previous study done by (Mutinda, 2016) with some adjustment. In this study, 20 chicks were proposed per experimental group, but in the current study, the experimental group was adjusted to 30 chicks to compensate death if occurred. Regardless of the sex chicks were randomly assigned into two (2) groups of 30 chicks. Group one was vaccinated and group two was not vaccinated that served as controls.

Blood was collected from wing vein of the chicks at specific intervals up to day 42. The chicken was handled by positioning it horizontally on its back, one hand hold the legs and the wing to support the chicken, the other hand was used to draw the blood. Sterile plastic disposable syringes of 2 mL and 29 gauges were used to collect blood. About 0.5 mL of blood was taken from each chick at day 1^{st}, 14^{th}, 21^{st}, 28^{th}, 35^{th} and 42^{nd} of age to obtain the sera. Blood samples were left to clot at room temperature overnight. Each blood sample was centrifuged at 3 000 rpm for 15 minutes to separate and obtain the sera. The serum was

harvested by using pasteur pipette and transferred into eppendorf tubes. All the sera were stored at -20 °C until analysis.

The vaccine used in this study was Virgo 7 strain (Biovac Limited, Israel). This is a live, an intermediate plus vaccine. Every dose contains about $10^{2.5}$ EID50. Virgo 7 strain vaccine was selected in this study for the reason that, the majority of poultry farmers in Tanzania use it for routine vaccination against IBDV. The vaccination requirements including handling, storage, schedule, reconstitution and administration were observed during the study. The vaccine was given to chicks at day 14 of age via drinking water as per manufacturer's recommendation. Blood sampling was done at day 1, 14, 21, 28, 35 and 42 to monitor the antibody titres. About 0.5 mL of blood was collected per chick per sampling.

Serological analysis of the study samples was done by ELISA technique using indirect ELISA kits (ID Vet, France) as per manufacturer's instructions. The ELISA kit reagents and serum samples that were stored at 2 – 8 °C and at -20 °C respectively were allowed to thaw at room temperature prior to analysis. An aliquot (5 µL) of each sample were added to the microtitre plate. Then 245 µL of dilution buffer was added to all wells except to control well A1, B1, C1 and D1. That gave a 1:50 dilution of a serum sample to diluents in the dilution plate. After that, 384 µL of negative control was added to well A1 and B1 and 384 µL of positive control were added to well C1 and D1 of the ID.vet test kit plate. Thereafter, 90 µL of dilution buffer was added to all wells except to control well A1, B1, C1 and D1. Then 10 µL of the pre-diluted samples were added to each corresponding well of the ID.vet test kit plate. This gave 384 µL per well and final dilution of 1:500 in dilution buffer. The plate was covered and incubated for 30 minutes at room temperature (25 – 27 °C). After 30 minutes the plate was emptied and washed three times with 300 µL of wash solution. The plate was then inverted and tapped firmly on absorbent paper. Then 384 µL of the conjugate was added to each well. The plate was covered and incubated for 30 minutes at room temperature. After 30 minutes the plate was emptied and washed 3 times with 300 µL of wash solution. The plate was inverted and tapped firmly on absorbent paper to dry. Then 384 µL of substrate solution was added to each well. The plate was covered and incubated for 15 minutes at room temperature. After that 384 µL of stop solution was added to each well to stop the reaction. The absorbance values were read and recorded at wavelength 450 nm using ELISA reader (Multiscan^{TM} FC Microplate Photometer).

The antibody titer in chicken serum sample was calculated by referring to the positive control. That association was expressed as S/P ratio (Sample to Positive Ratio). To confirm if the test results are valid the mean optical density (OD) value of the positive control should be greater than 0.250 and the ratio of the mean values of the positive and negative controls should be greater than 3.0. Interpretation of the results was done based on the S/P ratio and ELISA antibody titer. The result is positive if the S/P ratio is > 0.3 or antibody titer > 875 and result was considered negative when the S/P ratio is ≤ 0.3 or antibody titer ≤ 875.

3.7 Characterization of Field IBDV Strains and the Vaccine Strains Currently Used

The IBDV samples used in this study were taken from the repository based at Sokoine University of Agriculture (SUA). The samples were obtained during IBD outbreaks in Morogoro (n = 2) and Dar es Salaam (n = 1) between 2016 and 2018. Two commercial live IBDV vaccines namely; Virgo 7 intermediate strain (Biovac Ltd, Israel) and Globivac-Intermediate plus strain (Globin, India) were purchased from the veterinary centres located in the study area.

3.7.1 IBDV RNA Extraction

The process was done by extracting viral RNA directly from the bursa samples obtained in the field and vaccines using RNeasy Min Extraction Kit (QIAGEN GmbH, Germany). The extraction was done as per the manufacturer's instruction. Briefly, 460 µL of prepared lysis Buffer RLT was mixed with 460 µL of the viral sample in 1.5 micro centrifuge tube thereafter the mixture was incubated for 5 minutes at room temperature. Then 460 µL and 70% ethanol was added to the mixture and mixed by vortexing for 15 seconds.

The mixture was transferred into RNeasy spin column and centrifuged at full speed of 12 000 rpm; 10 000 g for 15 seconds. The flow-through was discarded. The spin column was washed with 700 µL wash buffer RW1 and centrifuged for 10 seconds at 10 000 rpm. It was then washed with 500 µL wash buffer RPE and the flow-through was discarded.

The washing was repeated with 500 µL using buffer RPE and centrifuged at full speed of 12 000 rpm for 2 minutes to dry membrane. The old collection tube was discarded and the column was transferred to a new collection tube and centrifuged at maximum speed for 1 minute to remove any traces of ethanol. Viral RNA was eluted with 50 µL nuclease-free

water into a new 1.5 mL tube. The obtained viral RNA was stored in a freezer at -20 °C. The tubes were clearly labeled and awaiting for reverse transcription process (cDNA synthesis).

3.7.2 cDNA Synthesis (RevertAid First Strand cDNA)

The complementary deoxyribonucleic acid (cDNA) strand was manufactured by using commercially available cDNA kit (RevertAid First Strand cDNA Synthesis Kit) manufactured by Thermo Scientific, Lithuania EU. The process contained the following reagents; Nuclease free water, extracted double-stranded RNA, Dimethyl sulfoxide (DMSO), reaction buffer, dNTPs, random hexamer primer, RNase inhibitor and reverse transcriptase enzyme (RevertAid RT). The process started by mixing 3 µL of each extracted dsRNA with 1.5 µL of DMSO and incubated at 97 °C for 5 minutes then immediately chilled on ice. The RT master mix which contains the reagent mentioned in Table 1 was added to the tube containing RNA-DMSO mixture to the final volume of 22.5 µL. The mixture was thoroughly mixed and the tubes were then sealed and centrifuged to settle down the contents and remove all bubbles. The sample mixture was then subjected into thermal cycler under the following conditions; 25 °C for 10 minutes, 37 °C for 120 minutes and 85 °C for 5 minutes. The synthesized cDNA was kept at 4 °C and used as a template for PCR.

Table 1: Master Mix for Reverse Transcription

No	Component	Volume (µL)
1	Reaction Buffer	4
2	Ribolock	1
3	Random Primer	1
4	Revert Aid	1
5	dNTP’s	2
6	Nuclease free water	9
	Total volume per reaction	18

3.7.3 Polymerase Chain Reaction

Polymerase chain reaction (PCR) was conducted by targeting the VP2 hyper-variable regions (HVRs) of IBDV through gene-specific V1 forward primer (5-CCA GAG TCT ACA CCA TAA-3) and V2 reverse primer (3-CCT GTT GCC ACT CTT TCG TA) (Yamaguchi *et al.*, 1996). The process was done by using PCR readymade Kit (Bioneer Corporation, South Korea). Briefly, 12.5 µL of PCR premix, 2 µL of primers and 14 µL of nuclease-free water were added to the PCR tube containing reverse transcriptase (RT) product under ice

condition. The mixture was then loaded in the PCR machine for amplification. The amplification reaction involved initial denaturation at 97 °C for 5 minutes, followed by 35 thermal cycles of denaturation at 94 °C for 30 seconds, annealing at 57 °C for 30 seconds, elongation at 72 °C for 30 seconds and final elongation step at 72 °C for 5 minutes and hold at 4 °C overnight.

3.7.4 Electrophoresis

This process was done in order to visualize the obtained PCR products. The electrophoresis of the PCR product was done by first preparing the agarose gel. Briefly, the agarose gel was prepared by mixing 1.2 g of agarose powder with 384 mL of tris-borate-EDTA (TBE) reagent in a heat resistant conical flask. The mixture was then boiled using a hot plate with a magnetic stirrer to get a clear solution. The clear solution was left to cool at room temperature until it reaches a temperature of 50 °C. Then, 3 µL of Gel red was added and swirled gently until the stain is clearly mixed. Casting tray was prepared by adding the appropriate combs on the tray and carefully pours the melted agarose solution on the casting tray. The tray with the gel solution was left for 45 minutes for the agarose gel to polymerize. The casting tray was placed in an electrophoretic chamber and TBE was added in an electrophoretic chamber until the gel was submerged. The combs were removed slowly and carefully to avoid breaking of the solidified gel.

Five microliters of PCR products were mixed with 2 µL of loading dye. Thereafter, the mixture was loaded in the electrophoresis wells and stained with 3 µL Gel red. The reaction was carried out at 384 V for 45 minutes. Ultraviolet transilluminator was used for visualization of DNA bands. The gel picture was taken for further documentation.

3.7.5 PCR Product Purification

The obtained DNA amplicons were purified as per the GFX PCR purification protocol to get pure DNA. The DNA was purified from dNTPs, unused primers, salts and DNA polymerase which were used during PCR reaction. Briefly, the process involved mixing of 5 µL of captured buffer and 1 µL of PCR sample. Then a GFX spin column was introduced into 2 mL collection tube. The samples were added to the GFX column and centrifuged for 60 seconds. This is to ensure that the DNA binds into the column. The filtrate was discarded and the GFX column was taken back in the same tube. The GFX column was washed with 0.5 mL of wash

buffer type 1 and centrifuged at 13 000 rpm for 60 seconds. After that, the GFX column was placed in a clean 1.5 mL micro centrifuge tube. To elute DNA, 20 µL elution buffer type 6 was added to the centre of the GFX membrane. The column was left to stand for 1 minute and then centrifuged at 13 000 rpm for 1 minute.

3.7.6 Cycle Sequence

Purified DNA product was subjected to cycle sequencing by using Big Dye Terminator Kit. The process for one sample was done by mixing 3.5 µL of water, 2 µL of a buffer, 0.5 µL of Big Dye, 3 µL of primer and 1 µL of DNA. The required volume for six samples used in the study as indicated in (Table 2). The process was carried out under the following conditions; 96 °C for 1 minute in one cycle, (96 °C for 10 seconds, 50 °C for 0.05 seconds and 60 °C for 4 minutes) for 25 cycles and finally stored at 10 °C till analysis.

Table 2: Master Mix for Cycle Sequence

No	Component	Volume (µL) × 1	Volume (µL) × 6
1	Nuclease free water	3.5	21
2	Buffer	2	12
3	Big Dye	0.5	3
4	Primer	3	18
5	DNA	1	6
	Total volume	10	60

The product obtained from the cycle sequence was purified by ethanol precipitation. Ethanol precipitation was done by adding 5 µL 125 mM EDTA and 60 µL 384% ethanol to each purified DNA product. The mixture in the tube was vortexed and left in the darkroom for 15 minutes and centrifuged at 13 000 rpm for 30 minutes. The supernatant was eliminated. Sixty microliters of 70% ethanol was added to the mixture and vortexed for 15 minutes followed by removal of all supernatant. Vacu-dry was done for 15 minutes in the dark and followed by addition of 20 µL Hi-Di formamide and then loaded in the sequencing machine.

3.7.7 Nucleotide Sequencing and Phylogenetic Analysis

Sequencing was done at the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture by using AB 3 500 Genetic analyzer. The sequencing was

conducted as per (Sanger *et al.*, 1977). The obtained nucleotide sequences were arranged and corrected to remove the primer binding part and the low-quality portion and retain the sequence quality of more than 95% by using Genius software, version 4.0.10. Confirmation of identity and homology of trimmed and edited sequence with the already uploaded sequences were done by using BLAST (Basic Local Alignment Search tool) on the NCBI website. Molecular evolutionary genetics analysis (MEGA) version 7 was used to perform the phylogenetic analysis of the study samples. The accuracy of the phylogenetic tree was calculated from the bootstrap values by using the Kimura two-parameter option with 3 840 bootstrap replicates created by the neighbour-joining (NJ) method (Kimura, 1980). To build a phylogenetic tree, nucleotide sequences of the study samples (strain PDSM18, MMRG16, RMRG19, Virgo 7 strain and Globivac) and VP2 gene sequences from 32 IBDV strains with various genotypes that were taken from GenBank were used as shown in Fig. 1. The accession number for the study samples and the reference strains obtained from GenBank are shown in appendix 5.

3.8 Statistical Analysis

The data collected during the study were processed using Microsoft Excel 2019 and then imported into statistical package for social science (SPSS) version 20. Frequencies for all the variables were calculated, while means and standard deviations were computed for the continuous variables. Knowledge, attitude and practices were computed by adding the scores from the variables regarding the respective score. Chi-square test and simple linear regression were used to measure the association between several factors. P-value < 0.05 with 95% CI was considered to be significant.

Calculation of the S/P ratio and the antibody titer was done by Microsoft Excel 2019. Data for serology were stored in Microsoft Excel 2019 before exported to SPSS for descriptive statistics. One-Way Analysis of Variance (ANOVA) was used to assess the differences in mean titers at each sampling time and the overall.

The efficacy of the vaccine was assessed based on the results of serologic assays (titers post-vaccination antibodies and the number of positive chickens). A Student t-test was used for comparison of antibody titer between vaccinated and non-vaccinated groups.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Demographic Characteristics of Study Participants

The socio-demographic characteristics of the study participants are presented in Table 3. A total of 384 poultry farmers in Dar es Salaam city were interviewed with more than half (66%) of the respondents being females who proportionally owned more flocks. The respondents had a mean age of 42 ± 9.3 years. Majority of the respondents (89%) were the owners of the project, the rest were the family member and attendants. Being the owner of their projects aided in the provision of right information regarding the control of the infectious bursal disease. The flock size varied between 170 and 4 000 chickens per household. More than half (53%) of the poultry farmers had between 5 to 8 years of poultry farming experience with the mean years of experience being 5.20 ± 3.14 years.

About 71% of the respondents had secondary school education and above. Majority of the respondents were commercial poultry farmers (78%), followed by employees (13%) and others (9%). Of all the respondents interviewed, 73% observed to keep broilers, 21% layers and 6% local breeds.

Table 3: Demographic Characteristic of Study Participants

Variable	Frequency (n)	Percentage (%)
Gender		
Male	131	34
Female	253	66
Age		
20-25	20	5
26-45	207	54
46+	157	41
Level of education		
Primary	111	29
Secondary	176	46
Certificate	53	14
Diploma	30	8
Degree	15	3
Marital status		
Married	326	85
Unmarried	58	15
Number of chickens		
170-300	42	11
301-900	157	41
901+	185	48
Types of chickens		
Broiler	280	73
Layers	80	21
Local	24	6
Position of respondent		
Owner	341	89
Attendant	34	9
Family member	9	2
Occupation		
Poultry keepers	299	78
Employed	49	13
Others	36	9

4.1.2 Respondent's Knowledge on IBD

Majority of the respondent (91%) heard of infectious bursal disease (IBD). Results revealed that among 91% respondents who have heard of IBD previously, 40% heard from veterinary professionals, 31% and 29% of them had observed the disease at their homes and neighbours, respectively. Although 91% of the respondents heard about the disease, 65% were not aware

of the causative agent. In addition, a small proportion (14%) of the respondents knew the IBD mode of transmission with clinical signs such as white watery diarrhoea, leg paralysis and sudden death frequently reported.

Concerning sign and symptom of IBD, only 36% of respondents knew the clinical signs of IBD. Among the clinical signs mentioned, white watery diarrhoea (82%) was the frequently responded answer, followed by leg paralysis (15%) and sudden death (5%). The majority (91%) of the respondents knew that vaccine for IBD is available and the control of IBD is possible. Nearly two-thirds of the respondents (64%) knew that not all age groups of chicken can be affected by IBD (Table 4).

Generally, the respondent appeared to have poor knowledge toward IBD on the causative agent, mode of transmission and symptoms. Furthermore, gender (p=0.024), education (p=0.014), flock size (p=0.021) and time for the keeping of chickens (p=0.01) had a significant influence on poultry keeper's IBD knowledge. Age of respondents had no significant association to the knowledge on IBD since at t-value 1.188, df 382, *P*=0.238.

Table 4: Knowledge of Respondents toward IBD in Dar es Salaam Region

Variable	Frequency (n)	Percentage (%)
Have you heard about IBD		
Yes	349	91
No	35	9
Where did you hear		
Veterinarian	140	40
Happened on my farm	108	31
Neighbors	101	29
Causes of IBD		
I don't know	227	65
Virus	122	35
Do you know the mode of transmission		
No	300	86
Yes	49	14
Do you know the clinical signs of IBD		
No	227	65
Yes	122	35
Signs and symptoms reported		
White watery diarrhea	286	82
Leg paralysis	45	13
Sudden death	18	5

4.1.3 Respondent's Attitude Which Contribute to IBD Outbreaks

All (100%) respondents agreed that maintaining a high level of bio-security measure help in preventing the spread of IBD. Majority (86%) of the respondents believe that IBD can be prevented by vaccination and 14% were neutral. A high proportion (89%) of respondents reported that proper vaccine handling including cold chain maintenance is important for vaccine effectiveness. About two-thirds (66%) of respondents did not know if the presence of disinfectants in water interferes with vaccine function (Table 5).

Generally, the respondents had a positive attitude toward IBD with a mean score of 4.68 ± 1.18 out of 6. On top of that, gender ($p = 0.01$), education ($p = 0.03$), flock size ($p = 0.02$) and time for the keeping of chickens ($p = 0.01$) have a significant influence on poultry keeper's attitude toward IBD.

Table 5: Attitude of the Respondent about IBD in Dar es Salaam Region

Variable	Frequency (n)	Percentage (%)
Good bio-security measures prevent IBD spread		
Agree	384	100
Neutral	0	0
Disagree	0	0
IBD can be prevented by vaccination		
Agree	330	86
Neutral	54	14
Disagree	0	0
Cold chain maintenance is important for vaccine		
Yes	341	89
No	0	0
I don't know	43	11
Presence of disinfectants in water interfere vaccine Function		
Yes	139	34
No	0	0
I don't know	245	66
Is IBD killer disease in chicken		
Agree	227	59
Disagree	0	0
Neutral	157	41
Requested more information on IBD		
Yes	384	100
No	0	0

4.1.4 Respondent's Practices Which May Contribute to IBD Vaccines Failure

Despite the fact that all respondents did routine cleaning, only 50% clean on a weekly basis, followed by 20% who did once per batch and 30% without a routine schedule for cleanness.

Based on vaccine handling, all respondents admitted that vaccines are usually packed in a plastic bag with an ice pack for transport from veterinary shops to farms. Although 91% of respondents vaccinate against IBD, 19% experience IBD outbreaks (Table 6). The 90% of respondents who experienced IBD outbreaks after vaccination did not adhere to the entire recommended procedures for vaccine reconstitution, handling and bio-security measures. More than two-thirds (78%) of respondents vaccinate their chickens once using IBD vaccine and 22% vaccinate twice. It was also observed that water from different sources was used to reconstitute the IBD vaccine such as tap water (50%), well water (48%) and tap water mixed with skimmed milk (2%). Only 31% of the respondents placed a foot bath on the entrance of the poultry house.

Table 6: Practices Related to IBD Management in Dar es Salaam Region

Variable	Frequency (n)	Percentage (%)
Disinfection of poultry house and equipment		
Yes	384	100
No	0	0
How often do you clean the chicken house, feeder and drinkers		
Every week	192	50
Once per batch	77	20
No specific schedule	115	30
Do you have a foot bath on the entrance of the poultry house?		
Yes	119	31
No	265	69
Do you vaccinate your chicken		
Yes	349	91
No	35	9
Vaccination regimen for IBD?		
Once	272	78
Twice	77	22
Others	0	0
Which water do you use in reconstituting the vaccine?		
Tap water	174	50
Well water	167	48
Tape water + milk	8	2
Have you ever experience IBD after vaccinating the chickens		
Yes	70	20
No	279	80

4.1.5 Relationship between Knowledge, Attitude and Practices Scores

The simple linear regression analysis revealed that there is an association between knowledge and practice ($t = 2.404$, $p = 0.02$), knowledge and attitude ($t = 13.201$, $p < 0.01$) and attitude and practice ($t = 2.575$, $p = 0.01$). The results confirm a relationship between knowledge, attitude and practices with regard to IBD management.

4.1.6 Handling of a Vaccine in Veterinary Shops

All the 20 veterinary shop owners who were interviewed reported packing vaccines in plastic bags with ice when they sell to their customers. Fourteen out of 20 shops observed to have standby generators. It was noted that the majority (14) of the shop operators lacked formal

training in handling of vaccines especially the maintenance of cold chain. Written procedures for vaccine handling and packaging into vaccine carriers was not available in all veterinary shops surveyed. There was neither temperature recording chart nor thermometer for monitoring the temperature in all the veterinary shops visited.

4.1.7 Decreasing Trend of Maternally Derived Antibodies

Based on ELISA results the mean value of maternally derived antibodies (MDA) decline rapidly from day 1 (2 931 ± 196.6) up to day 14 (1 297 ± 122.2) and thereafter progressive declining up to (196.5 ± 54.82) at day 42. The highest mean of MDA (2 931 ± 196.6) was recorded on day 1 of age.

Significant differences ($p = 0.00$) in MDA titers at day 1, 14 and 42 were observed when compared by one-way analysis of variance (ANOVA) and this demonstrate that the MDA titers decreased from day to day and reach a level (196.5 ± 54.82) to which it cannot protect the chicks.

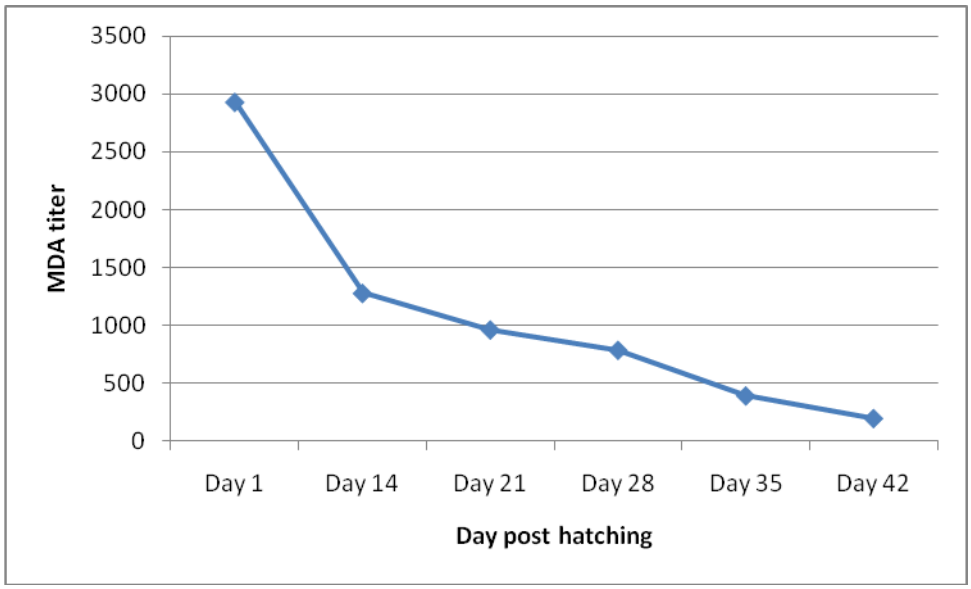

Figure 1: Decaying Pattern of MDA with Age of Chickens

4.1.8 Immunogenicity of Virgo 7 Strain Vaccine

Following the administration of the Virgo 7 strain vaccine, the mean serum antibody titers in the vaccinated group started to increase which peaked on 3rd week (day 35 of age). Compared to unvaccinated group, the mean serum antibody titers in the vaccinated group were significantly higher ($p < 0.05$) from day 14, all through day 35 (Fig. 2). Antibody titers in

control group progressively decreased and were at lowest levels on day 42 when the experiment was terminated. This was due to the diminishing of maternal antibodies.

The variation of mean antibody titer between age group in vaccinated chickens was analyzed by one way ANOVA and found to be statistically significant ($p = 0.0001$). Furthermore, titers from vaccinated and non-vaccinated groups were compared using paired independent t-test and the result shows a significant variation of antibody titers ($p < 0.005$) at 21st, 28th, 35thand 42nd day.

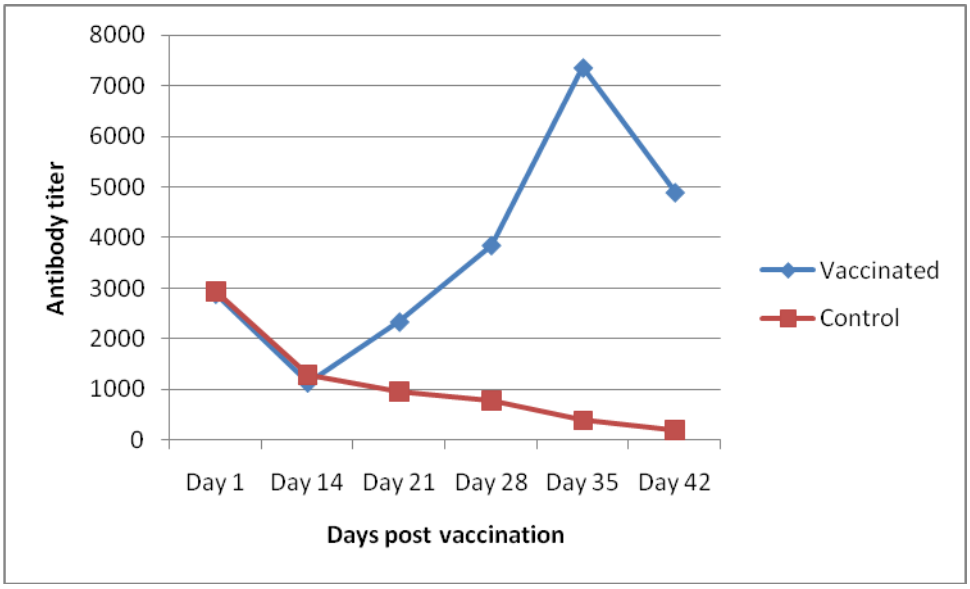

Figure 2: Antibody Titer after Vaccination Compared to Unvaccinated Controls

4.1.9 Extraction and Amplification of the IBDV Gene from the Field Samples and Vaccines

The results show reliable amplicons IBDV VP2 HVR gene for 3 field samples (PDSM18, MMRG16 and RMRG19) and 2 imported IBDV vaccines (Virgo 7 strain and Globivac) after conducting PCR.

4.1.10 BLAST Search Analysis of VP2

After blasting the nucleotide sequences of the study samples, it was observed that the nucleotide sequences of 2 Tanzanian IBDV field samples (PDSM18 and MMRG16) were highly similar (94.68 - 96.33% nucleotide sequence identity) to a virus (LUSC47-2016) detected in Zambia 2016. Sample RMRG19 showed 99.53% nucleotide sequence identity with JG028/KEN/16 isolated in Kenya. Globivac vaccine showed 99.34% nucleotide sequence identity with D78 isolated in the USA. However, the Virgo 7 strain vaccine showed

100% nucleotide sequence identity with strain IBD10HLJ02 isolated in China, 713_Russia isolated in Russia, 213-048-2 isolated in Brazil, West Bengal/HBL-07-15-b isolated in India and V90/TW95 isolated in Taiwan.

4.1.11 Phylogenetic Analysis

Phylogenetic analysis using the HVR of the VP2 gene of the current IBDV samples and the representative strains from GenBank was performed to compare and verify the evolutionary lineage of the study samples. The phylogenetic tree revealed that the IBDV samples were separated into two main groups namely; Classical attenuated and very virulent (vv) viruses (Fig. 3). The very virulent (VV) IBDVs further divided into two major groups namely; VV1 and VV2. The VV1 contains strains isolated from Africa including Tanzania, Zambia, Kenya and Nigeria which called vv IBDV African genotype, while VV2 contains strains isolated from various continents of the world including Africa, Asia America and Europe which form vvIBDV European genotype. The current field strains (RMRG19, PDSM18 and MMRG16) were grouped in the VV1 cluster while the imported Virgo 7 strain vaccine were grouped in VV2 and Globivac vaccine was grouped in classical virulent type (Fig. 3).

Phylogenetic tree based upon nucleotides of segment A showed that the field IBDV strain PDSM18 and MMRG16 are closely related (96% bootstrap value) with vvIBDV African type (LUSC47-2016). The field strain RMRG19 was more associated (66% bootstrap value) with both vvIBDV African types (JG028/KEN/16 and JG026/KEN/16). The imported Virgo 7 strain vaccine was genetically closely related (97% bootstrap value) to the vvIBDV European/Asian type JG011/KEN/15, 213-048-2/2017, BD10HLJ02, 13_Russia-2017, V90/TW95 and IBDV78/ABIC. The imported Globivac vaccine was genetically related to (100% bootstrap value) classical virulent (D78, 534_North_Carolina, 03-27950-dn and BD07IR). The sequence analyses indicated that the field strains were genetically different from the vaccine strains present in the market for IBD control.

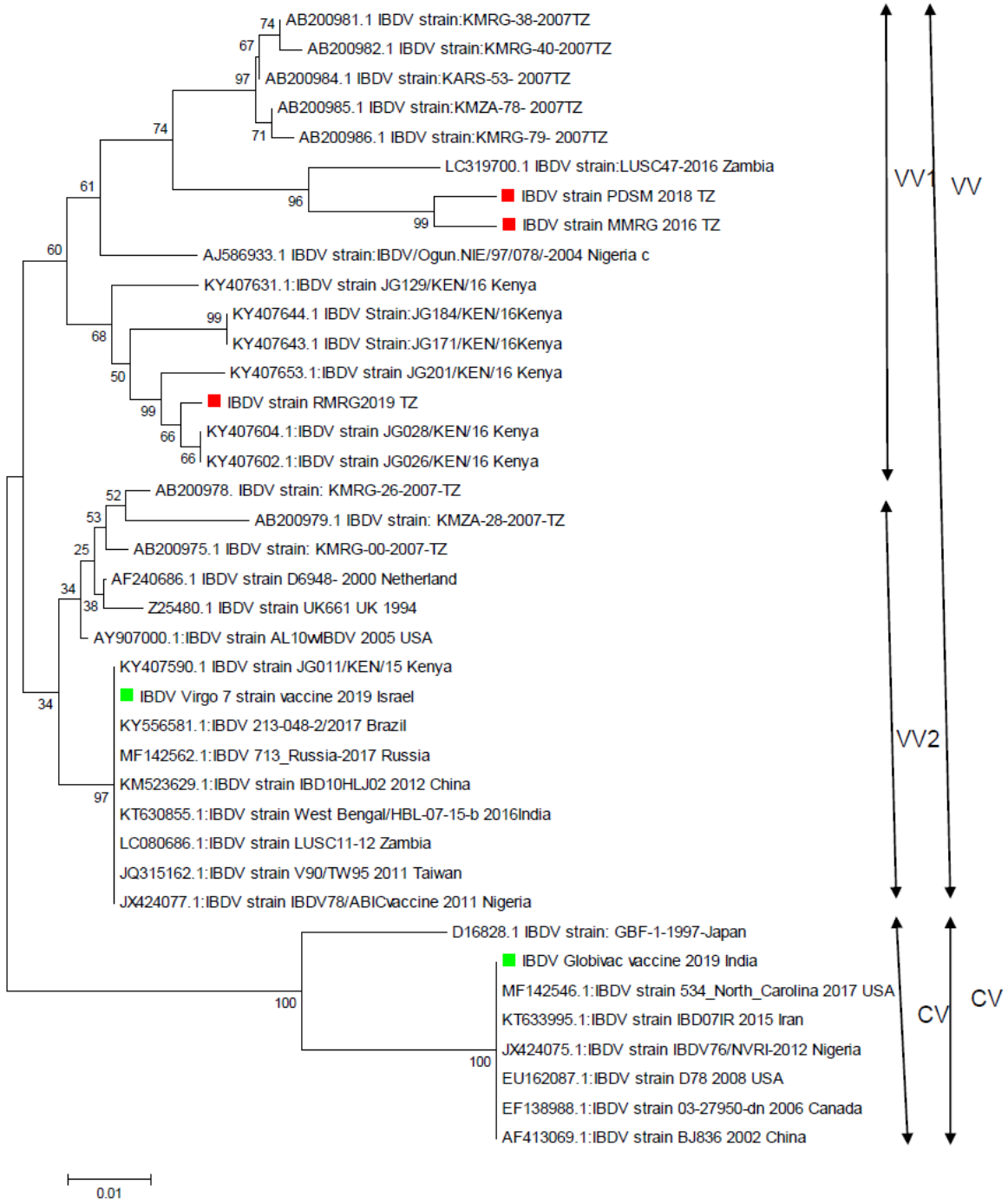

Figure 3: Phylogenetic Tree of 3 Fields IBDV Isolates, Reference Strains and Vaccines.

Note: VV: Very virulent genotype, VV1: Very virulent African type; VV2: Very virulent European/Asian type; CV: Classical and antigenic variant genotype; Field isolate from Tanzania are in red; imported vaccines used in Tanzania are in green; reference strains obtained from GenBank not marked.

4.2 Discussion

Infectious bursal disease is very devastating disease in the poultry production as a result of mortality and exposing chickens to be more susceptible to secondary infections (Kurukulasuriya, 2017). The disease is more severe in Africa since the vaccines used are apparently not protective against emerging IBDV field strains (Mohamed *et al.*, 2014) and inadequate bio-security measures. In Tanzania, some poultry farmers have encountered IBD outbreaks in both vaccinated and non vaccinated chickens, this indicating that there is a possibility of vaccine inefficiency or failure (Kasanga *et al.*, 2007). Therefore, the current study was performed to examine the possible causes of vaccination failure including assessment of immunogenic potential of the Virgo 7 strain IBD vaccine in chicks, knowledge, attitude and practices of poultry farmers and vaccine seller and phylogenetic relationship between circulating IBD virus strains in the fields and imported infectious bursal disease virus vaccine strains.

To evaluate IBD knowledge of the poultry farmers, structured questionnaire was used. Results demonstrated that majority of poultry farmers had heard about infectious bursal disease in their locality. This demonstrates that IBD continue to be a most important threat of poultry production in the study area. Despite the majority of farmers having heard about the disease, knowledge concerning IBD is very low especially on causes, transmission and clinical signs similar to reports from other countries (Chawinga, 2016; Radostits *et al.*, 2000). The knowledge gap identified in farmers with regard to causes, signs, and transmission against IBD is an indication of poor poultry extension services on IBD management. Therefore there is a need of strengthening poultry extension services and training to poultry farmers on disease management especially IBD. These extension services and training will improve farmer's knowledge and subsequently lower IBD transmission rates.

In contrast, the study conducted by Wahome (2018) reported a high level of knowledge and awareness about IBD among poultry keepers in Embu county, Kenya. The variation in awareness and knowledge on the clinical presentation of IBD, transmission and causes could be attributed to the endemic rate of IBD in the community, presence of veterinary services, formal training to poultry farmers and education through other media in Embu county, Kenya. Those few farmers who had fair knowledge on IBD could be due to their habits of

consulting veterinary officers whenever they see problems in their flocks and being exposed to the Medias where they get information on several diseases.

Even though knowledge of IBD was low among poultry farmers, level of education found to influence farmers knowledge against IBD. Those with high level of education were observed to be somehow knowledgeable compared to those with low level of education. This could be due to the fact that educated farmers are more likely to understand disease management practices.

Flock size and duration of keeping chickens also reported to influence farmer's knowledge against IBD in this study. This could be due to the fact that, when farmers keep a large number of chickens they are more likely to consult their neighbours and veterinary personnel before vaccination and when there is disease outbreak due fear of loss. Furthermore, when farmers keep chickens for a long time they are most likely to encounter a number of diseases so they become aware of so many diseases.

A structured questionnaire was also used to assess the practices of the poultry farmers and the results showed that 91% of respondents vaccinate their chickens against IBD. The findings were similar to the study conducted in Nigeria which showed that 84.1% of respondents vaccinated their birds (Bosha & Nongo, 2012). Furthermore, the study showed that 80% of famers who vaccinated their chickens against IBD did not experience disease outbreak. In this regard, it can be considered that the vaccines currently used in Tanzania might protect chickens against some field IBDV strains. The 20% vaccination failure observed in the study might be due to the consequences of violations of the prescribed good vaccine handling practices and administration demonstrated by some farmers which include poor sanitation, non-adherence to cold chain system, vaccine preparation and administration. Another explanation could be the IBDV strain affecting these farms is the mutated strain of IBDV isolated recently in this study (very virulent African type) which is genetically different from vaccine strains. Therefore, more education with regard to IBD management and vaccine handling and application should be provided to farmers so that we can reduce the cases of vaccination failure to zero. Furthermore, regular molecular monitoring of IBDV evolution is required in order to understand the dynamics of IBDV strains in Tanzania, since it is critical for control of IBD in the country.

The majority of respondents reported that during vaccine reconstitution they use tap water that contains chlorine although IBD vaccine manufacturers recommended the use of chlorine-free water or if doubtful add 2 g/L of skim milk. Addition of skimmed milk in water used for vaccination is very important as it help to prevent the harmful effect of chlorine and other disinfectants that might impinge on the efficiency of vaccine. Therefore, the practice of using potable water by most of the poultry farmers in the study area might be one of the causes for observed incidences of vaccination failure. This high rate of farmers using chlorinated water in vaccine reconstitution was also reported by Isegbe (2014).

The present study also assessed the status of cold chain in veterinary outlets in Dar es Salaam region by using a checklist. The results showed that there was lack of functional thermometer in all veterinary shops surveyed. As per the vaccine logistic management guidelines, thermometer must be available in any vaccine storage center to record maximum and minimum temperature. This guarantee vaccine potency and the success of immunization, when it is not available at vaccine storage centers that means such centers do not monitor their refrigerator temperature and in that case the cold chain can easily be interrupted. The interruption of cold chain might affect the antigenicity of the vaccine hence result to vaccination failure. The finding is in agreement with (Azira *et al.*, 2014; Simba & Msamanga, 1994) who emphasized temperature monitoring in vaccine centers to ensure vaccines are of acceptable quality until administration to recipients.

Another issue of serious concern was availability of power. Some of the outlets visited did not have backup generator in case of power outage. This give an indication that cold chain maintenance may be interrupted at some point and exposing the vaccines to unfavorable temperature. It should be noted that electricity or an alternative source of power is essential for cold chain maintenance as high temperature may cause death of viruses and affect the viability of antigenic material as reported by Bosha and Nongo (2012) and subsequently cause vaccine failure.

The study revealed a high level of maternally derived antibodies (MDA) titers which were above the minimum (2 931 ± 196.6) protective level in the day-old chicks. This level was maintained up to day 7 and then decreased subsequently. High level of MDA noted in the present study implies that the parent stock was hyperimmunised and passed on a high level of antibodies to the progeny. This high level of MDA is very important since it provides

protection of chickens at an early age when they are more susceptible to IBDV infection. On the other hand, high level of MDA need to be considered especially when implementing vaccination against IBD since high MDA titer at early age may interfere with vaccine through neutralization (Sarachai *et al.*, 2010) and thus recommended to vaccinate when the MDA titers waned out below protection level. Based on the current study, chicks from breeder farms in Tanzania have higher MDA's up to (2 931 ± 196.6) but declined rapidly to 1 297 ± 122.2 after 14 days and continue to be detected in chickens up to day 42 with titer below protection level. With reference to the experimental study results it is recommended that vaccination will be successful if will be implemented after two weeks of age where MDA level will be below the protection level and less interference with live vaccines.

An experimental study was conducted to evaluate the ability of commonly used IBDV vaccine (Virgo 7 intermediate hot strain IBDV vaccine) to induce antibodies against IBDV following vaccination of chickens. The results showed that, Virgo 7 vaccine was capable of producing antibodies against IBDV in vaccinated chickens. Based on the results obtained in the experimental study, it is recommended that vaccination will be successful if will be implemented after two weeks of age where MDA level will be below the protection level and less interference with live vaccines. However, farmers and other stakeholders should note that vaccination time depends on the source of chickens and the type of vaccines used. Different breeder farms have a different level of MDA (Hassan *et al.*, 2018). Therefore, different vaccination schedule should be implemented. The current study has shown that, poultry farmers in Dar es Salaam use the same vaccination schedule regardless of the source and type of chicks. This might be one of the factors accounts for the incidences of IBD outbreaks in vaccinated chicken flocks. On the other hand, this study should be conducted on a wider scale so as to confirm the potency of other IBD vaccines being used in the country. Further study is also needed to examine the protective efficacy of this and other IBD vaccines being used in Tanzania.

Phylogenetic analysis of three field strains and two imported vaccines strains has been conducted to assess their genetic relatedness. Phylogenetic study of the deduced nucleotide sequences of the three Tanzanian field strains showed that all samples belong to the pathogenic serotype-1, whereas subgenotype analysis revealed that the samples belong to the vvIBDV African genotypes, which include strains from the west, east and southern Africa. The current Virgo 7 strain vaccine used in Tanzania belongs to the vvIBDV European

genotype while Globivac vaccine belonged to the classical strain. This indicates that, the vaccines used in the control of IBDV in Tanzania are genetically different from field isolate recently detected in Dar es Salaam and Morogoro and this may contribute to outbreaks in vaccinated flocks. This calls for further research on appropriate vaccines made using local IBD strains.

Furthermore, other studies have demonstrated that vaccination of chicken using vaccine made by classical virulent IBDV strains to prevent the disease caused by very virulent IBDV strains might cause exchange of genes between the two virus and resulting to a natural reassortant virus as reported in China, Zambia and Argentina (Fernandes *et al.*, 2012; Kasanga, 2015). In view of this, understanding the basis for evolutionary characteristics and genetic variation of field viruses could help veterinarians and researchers to design and develop new vaccines for controlling IBD, which cannot be controlled by current vaccines being used in the country.

Generally, vaccine failures have been reported globally and are attributed to several factors. Under the current study the observed attributed factors for vaccination failure were poor sanitation, lack of adherence to vaccination regimen, use of chlorinated water in vaccine reconstitution, cold chain breaks due to frequent power outage, lack of standby generator and using vaccines with different strains from circulating field strain in Tanzania. Those factors may compromise the vaccine efficacy and vaccination process hence resulted to reported cases of IBD outbreak in vaccinated chicken's flocks.

4.3 Limitation of the Study

There are limitations to our study that should not be overlooked when interpreting these findings. In establishing the efficacy of virgo 7 stains vaccine, antibody titer was used as the parameter for concluding efficacy of this vaccine. This has limitation since the antibodies produced might not be able to protect chickens against the field IBDV strains. Therefore, further studies are needed to investigate whether the induced antibodies by study vaccine are protective against Tanzania IBDV strains.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The success of vaccination programme depends on various factors such as vaccine composition, route of administration, timing in administering vaccine, the ability of the chickens to produce protective antibodies after vaccination and the adherence of vaccine handlers to general good vaccine handling practices and manufacturer's recommendation. In the current study, the knowledge of vaccine handling and administration was observed to be inadequate among both vaccine sellers and farmers. The present study pointed out that, there were a lot of breaches in the cold chain maintenance which may possibly jeopardize the efficacy of the vaccines and the overall quality of the vaccination services.

The current study confirmed that maternal antibodies against IBDV are passively transferred to the progeny. Therefore, early vaccination may cause neutralization of vaccine virus by circulating MDA. The current study proposed vaccination using Virgo 7 strain vaccine to be at day 14^{th} of age as proposed by vaccine manufacturer since the vaccine was able to produce antibodies against IBDV in vaccinated chickens. However, further studies are needed to investigate whether the induced antibodies by study vaccine are protective against Tanzania IBDV strains.

The current study discovered antigenic and genotypic differences between Tanzanian IBDV isolates and imported vaccine strains (Globivac and Virgo 7 strain). This study suggested considering of local virus isolates during vaccine development.

5.2 Recommendations

Poultry extension services should be strengthened to educate farmers and vaccine handlers (veterinary outlets) on the importance of observing all recommended vaccine protocol to minimize vaccine failure in the country.

It is recommended that, it should be mandatory for vaccine manufacturers to include a leaflet in "Swahili" and English language on how the vaccines should be handled.

Further research is recommended to address other factors that may influence time to vaccinate such as a source of chicks and type of vaccine and to confirm the potency of other IBD vaccines being used in the country apart from Virgo 7 strain vaccine.

Further investigation is warranted to establish transmission dynamics, evolutionary characteristics and antigenicity of IBDV in order to design appropriate control strategies.

REFERENCES

Abdullahi, U., Adamu, S., & Ahmed, A. (2009). Investigating on some causes of poultry vaccination failure in Bauchi Metropolis and environs, Nigeria. *Nigerian Journal of Experimental and Applied Biology, 14*(1), 47-50.

Ahmed, Z., & Akhter, S. (2003). Role of maternal antibodies in protection against infectious bursal disease in commercial broilers. *International Journal of Poultry Science, 2*(4), 251-255.

Al-Natour, M., Ward, L., Saif, Y. M., Stewart-Brown, B., & Keck, L. (2004). Effect of different levels of maternally derived antibodies on protection against infectious bursal disease virus. *Journal of Avian Diseases, 48*(1), 177-182.

Angani, M., Abdu, P., & Sa'idu, L. (2014). Evaluating the protective efficacy of a live and two killed vaccines against infectious bursal disease in commercial chicks in Zaria, Nigeria. *Journal of Experimental Biology and Agricultural Sciences, 2*(1), 59-63.

Aydin, S. (2015). A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. *Peptides, 72*(1), 4-15.

Azira, B., Norhayati, M., & Norwati, D. (2014). Optimal temperature of cold chain and its associated factors among general practitioners in Kelantan, Malaysia. *International Journal of Collaborative Research on Internal Medicine & Public Health, 6*(6), 168-172.

Becht, H., Müller, H., & Müller, H. K. (1988). Comparative studies on structural and antigenic properties of two serotypes of infectious bursal disease virus. *Journal of General Virology, 69*(3), 631-640.

Benton, W., Cover, M., & Rosenberger, J. (1967). Studies on the transmission of the infectious bursal agent (IBA) of chickens. *Journal of Avian Diseases, 11*(3), 430-438.

Berg, T. P. V. D. (2000). Acute infectious bursal disease in poultry: A review. *Journal of Avian Pathology, 29*(3), 175-194.

Birghan, C., Mundt, E., & Gorbalenya, A. E. (2000). A non-canonical Lon proteinase lacking the ATPase domain employs the Ser–Lys catalytic dyad to exercise broad control over the life cycle of a double-stranded RNA virus. *The EMBO Journal, 19*(1), 114-123. https://doi,org/10.1093/emboj/19.1.114

Bosha, J. A., Nongo, N. N., (2012). Common breaches in Poultry vaccine handling and administration in Makurdi Metropolis: A recurrent phenomenon in the Tropics. *Journal of Veterinary Science 9*(1),11-16.

Brown, F. (1986). The classification and nomenclature of viruses. Summary of results of the international committee on taxonomy of viruses in sendai, september 1984. *Journal of Intervirology, 25*(3), 141-143.

Cardenas-Garcia, S., Ferreri, L., Wan, Z., Carnaccini, S., Geiger, G., Obadan, A. O., Perez, D. R. (2019). Maternally-derived antibodies protect against challenge with highly pathogenic Avian influenza virus of the H7N3 subtype. *Journal of Vaccines, 7*(4), 163-164.

Chawinga, K. (2016). *Epidemiological study of infectious bursal disease virus in selected districts of the Copperbelt province in Zambia* (Masters Dissertation, Sokoine University of Agriculture, Tanzania). Institutional Repository at SUA. http://www.suaire.suanet.ac.tz:8080/xmlui/handle/123456789/1541

Ching Wu, C., Rubinelli, P., & Long Lin, T. (2007). Molecular detection and differentiation of infectious bursal disease virus. *Journal of Avian Diseases, 51*(2), 515-526.

Cosgrove, A. (1962). An apparently new disease of chickens: Avian nephrosis. *Journal of Avian Diseases, 6*(3), 385-389.

Dey, S., Pathak, D. C., Ramamurthy, N., Maity, H. K., & Chellappa, M. M. (2019). Infectious bursal disease virus in chickens: Prevalence, impact, and management strategies. *Journal of Veterinary Medicine, 10*(3), 85-97.

Eterradossi, N., Gauthier, C., Reda, I., Comte, S., Rivallan, G., Toquin, D., Morin, Y. (2004). Extensive antigenic changes in an atypical isolate of very virulent infectious bursal disease virus and experimental clinical control of this virus with an antigenically classical live vaccine. *Journal of Avian Pathology*, *33*(4), 423-431.

Eterradossi, N., & Saif, Y. (2008). Infectious bursal disease. *Journal of Diseases of Poultry, 12*(1), 185-208.

Fahey, K. J., Erny, K., & Crooks, J. (1989). A conformational immunogen on VP-2 of infectious bursal disease virus that induces virus-neutralizing antibodies that passively protect chickens. *Journal of General Virology*, *70*(6), 1473-1481.

Faulkner, O. B., Estevez, C., Yu, Q., & Suarez, D. L. (2013). Passive antibody transfer in chickens to model maternal antibody after avian influenza vaccination. *Journal of Veterinary Immunology and Immunopathology, 152*(4), 341-347.

Fernandes, M. J. B., Simoni, I. C., Harakava, R., Rivas, E. B., & Arns, C. W. (2012). Partial VP1 sequencing of Brazilian infectious bursal disease virus strains. *Brazilian Journal of Microbiology, 43*(3), 1015-1021.

Gharaibeh, S., & Mahmoud, K. (2013). Decay of maternal antibodies in broiler chickens. *Journal of Poultry Science*, *92*(9), 2333-2336.

Guittet, M., Picault, J., & Bennejean, G. (1982). Gumboro disease: Maternal immunity transmitted to chicks from vaacinated breeders. *Journal of Developments in Biological Standardization*, 51(2), 221-233.

Haddad, E., Whitfill, C., Avakian, A., Ricks, C., Andrews, P., Thoma, J., & Wakenell, P. (1997). Efficacy of a novel infectious bursal disease virus immune complex vaccine in broiler chickens. *Journal of Avian Diseases*, *41*(4), 882-889.

Hair-Bejo, M., Ng, M., & Ng, H. (2004). Day-old vaccination against the infectious bursal disease in broiler chickens. *International Journal of Poultry Science, 3*(2), 124-128.

Hanjeet, K., Lye, M., Sinniah, M., & Schnur, A. (1996). Evaluation of cold chain monitoring in Kelantan, Malaysia. *Bulletin of the World Health Organization, 74*(4), 391-397.

Hasan, A. R., Ali, M., Siddique, M., Rahman, M., & Islam, M. (2010). Clinical and laboratory diagnoses of newcastle and infectious bursal diseases of chickens. *Bangladesh Journal of Veterinary Medicine, 8*(2), 131-140.

Hassan, F., Abdul, P., Saidu, L., & Bawa, E. (2018). Maternal antibody titre as a monitoring tool for vaccination against infectious bursal disease. *Sokoto Journal of Veterinary Sciences, 16*(3), 18-23.

Heller, E., Leitner, H., Drabkin, N., & Melamed, D. (1990). Passive immunisation of chicks against Escherichia coli. *Avian Pathology, 19*(2), 345-354.

Helmboldt, C. F., & Garner, E. (1964). Experimentally induced Gumboro disease (IBA). *Journal of Avian Diseases, 8*(4), 561-575.

Hirai, K., Funakoshi, T., Nakai, T., & Shimakura, S. (1981). Sequential changes in the number of surface immunoglobulin-bearing B lymphocytes in infectious bursal disease virus-infected chickens. *Journal of Avian Diseases, 25*(2), 484-496.

Hitchner, S. (1976). Immunization of adult hens against infectious bursal disease virus. *Journal of Avian Diseases, 20*(3), 611-613.

Huff, G., Zheng, Q., Newberry, L., Huff, W., Balog, J., Rath, N., Skeeles, J. (2001). Viral and bacterial agents associated with the experimental transmission of infectious proventriculitis of broiler chickens. *Journal of Avian Diseases, 45*(4), 828-843.

Isegbe, E., Agbontale, A., Alonge, G., Eimunjeze, M., Unigwe, C., & Okorafor, U. Vaccine Handling and Administration Among Poultry Farmers in Ibadan Metropolis, Oyo State, Nigeria.*Journal of Agriculture and Veterinary Sciences, 1*(2), 69-74

Islam, M. N., Rahman, M. M., Akter, M. R., Khan, M. S. R., Salauddin, M., & Islam, M. H. (2019). Quantification of protective antibody vaccine-elicited in chickens against infectious bursal disease virus. *Journal of Agricultural Science and Technology A, 9*(20), 38-45.

Ismail, N., & Saif, Y. (1991). Immunogenicity of infectious bursal disease viruses in chickens. *Journal of Avian Diseases, 35*(3), 460-469.

Ismail, N., Saif, Y., & Moorhead, P. (1988). Lack of pathogenicity of five serotypes 2 infectious bursal disease viruses in chickens. *Journal of Avian Diseases*, *32*(4), 757-759.

Jackwood, D., Hanes, G., & Miller, S. H. (1996). Infectious bursal disease viral RNA amplification using RT/PCR from bursa tissue following phenol: Chloroform inactivation of the virus. *Journal of Avian Diseases, 40*(2), 457-460.

Jackwood, D. J., Saif, Y. M., & Hughes, J. H. (1982). Characteristics and serologic studies of two serotypes of infectious bursal disease virus in turkeys. *Journal of Avian Diseases, 26*(4), 871-882.

Jackwood, D. J., Sommer-Wagner, S. E., Stoute, S. T., Woolcock, P. R., Crossley, B. M., Hietala, S. K., & Charlton, B. R. (2009). Characteristics of a very virulent infectious bursal disease virus from California. *Journal of Avian Diseases, 53*(4), 592-600.

Kapaga, A., Msami, H., & Mella, P. (1989). Infectious bursal disease Gumboro disease in Tanzania, presented at the Proceedings of the 7th Tanzania Veterinary Association Scientific Conference held at AICC, Arusha, 2-5 December 1989. https://www.researchgate.net/publication/51557199

Kasanga, C., Yamaguchi, T., Wambura, P., Maeda-Machang'u, A., Ohya, K., & Fukushi, H. (2007). Molecular characterization of infectious bursal disease virus (IBDV): Diversity of very virulent IBDV in Tanzania. *Archives of Virology, 152*(4), 783-790.

Kasanga, C. J. (2015). Partial molecular characterization of infectious bursal disease virus detected in Africa: potential evidence for virus recombination and genome segments A and B reassortment in nature. *Research Opinions in Animal and Veterinary Sciences, 5*(12), 468-475.

Kim, I., Gagic, M., & Sharma, J. M. (1999). Recovery of antibody-producing ability and lymphocyte repopulation of bursal follicles in chickens exposed to infectious bursal disease virus. *Avian Diseases, 43*(3), 401-413.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution, 16*(2), 111-120.

Kish, L., Leslie, J. (1965). Survey Sampling. *Biometrical Journal*, *10*(1), 88-89.

Kurukulasuriya, S. N. (2017). *Efficacy of Vaccines Against Variant Infectious Bursal Disease Viruses to Control Immunosuppression in the Broiler Chicken Industry in Canada (*Doctoral Dissertation, University of Saskatchewan). Institutional Repository at Saskatchewan. http://hdl.handle.net/10388/7617

Lawal, J., Jajere, S., Bello, A., Mustapha, M., Wakil, Y., Ndahi, J., Ibrahim, U. (2014). Prevalence of infectious bursal disease (Gumboro) antibodies in village chickens in Gombe state, northeastern Nigeria. *International Journal of Poultry Science, 13*(12), 703-708.

Lejal, N., Da Costa, B., Huet, J., & Delmas, B. (2000). Role of Ser-652 and Lys-692 in the protease activity of infectious bursal disease virus VP4 and identification of its substrate cleavage sites. *Journal of General Virology, 81*(4), 983-992.

Lequin, R. M. (2005). Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay. *Clinical Chemistry, 51*(12), 2415-2418.

Liu, M., & Vakharia, V., (2006). Nonstructural protein of infectious bursal disease virus inhibits apoptosis at the early stage of virus infection. *Journal of Virology, 80*(7), 3369-3377.

Ma, H., Zhao, S., Ma, Y., Guo, X., Han, D., Jia, Y., Teng, K. (2013). Susceptibility of chicken Kupffer cells to Chinese virulent infectious bursal disease virus. *Journal of Veterinary Microbiology, 164*(4), 270-280.

Mahgoub, H. A. (2012). An overview of the infectious bursal disease. *Archives of Virology, 157*(11), 2047-2057.

Maraver, A., Ona, A., Abaitua, F., González, D., Clemente, R., Ruiz-Díaz, J. A., Rodriguez, J. F. (2003). The oligomerization domain of VP3, the scaffolding protein of infectious bursal disease virus, plays a critical role in capsid assembly. *Journal of Virology, 77*(11), 6438-6449.

Mbuko, I., Musa, W., Ibrahim, S., Saidu, L., Abdu, P., Oladele, S., & Kazeem, H. (2010). A retrospective analysis of infectious bursal disease diagnosed at poultry unit of Ahmadu Bello University, Nigeria. *International Journal of Poultry Science*, *9*(8), 784-790.

Meulemans, G. (2000). Infectious bursal disease (Gumboro disease). *Review of Science and Technology Office of International Epizootics, 19*(2), 527-543.

McFerran, J., McNulty, M., McKillop, E., Connor, T., McCracken, R., Collins, D., & Allan, G. (1980). Isolation and serological studies with infectious bursal disease viruses from fowl, turkeys and ducks: demonstration of a second serotype. *Avian Pathology, 9*(3), 395-404.

Mohabe, L. K. (2012). Studies on prevalence, pathology and molecular diagnosis of infectious bursal disease in broilers.*Chhattisgarh Kamdhenu Vishwavidyalaya, Durg*, *2*(1), 20-67.

Mohamed, M. A., Elzanaty, K. E., Bakhit, B. M., & Safwat, M. M. (2014). Genetic Characterization of Infectious Bursal Disease Viruses Associated with Gumboro Outbreaks in Commercial Broilers from Asyut Province, Egypt. *Journal of Veterinary Science, 3*(1), 2-9.

Mubito, E. P., Shahada, F., Kimanya, M. E., & Buza, J. J. (2014). Antimicrobial use in the poultry industry in Dar-es-Salaam, Tanzania and public health implications. *American Journal of Research Communication, 2*(1), 51-63.

Müller, H., Mundt, E., Eterradossi, N., & Islam, M. R. (2012). Current status of vaccines against the infectious bursal disease. *Avian Pathology*, *41*(2), 133-139.

Musa, I., Sa'idu, L., Adamu, J., Mbuko, I., Kaltungo, B., & Abdu, P. (2010). Outbreaks of Gumboro in growers in Zaria, Nigeria. *Nigerian Veterinary Journal*, *31*(4), 306-310.

Muskett, J., Reed, N., & Thornton, D. H. (1985). Increased virulence of an infectious bursal disease live virus vaccine after passage in chicks. *Vaccine, 3*(4), 309-312.

Mutinda, W. U. (2016). *Isolation, pathological and immunological characterization of Kenyan infectious bursal disease virus strains for vaccine development* (Doctoral Thesis, University of Nairobi, Kenya). Institutional Repository at Semanticscholar. https://pdfs.semanticscholar.org/e367/01eabe720a96e1803d985655a1f447a23452.pdf

Mutinda, W. U., Nyaga, P. N., Mbuthia, P. G., Bebora, L. C., & Muchemi, G. (2014). Risk factors associated with infectious bursal disease vaccination failures in broiler farms in Kenya. *Tropical Animal Health and Production, 46*(4), 603-608.

Naqi, S., Marquez, B., & Sahin, N. (1983). Maternal antibody and its effect on infectious bursal disease immunization. *Avian Diseases*, *27*(3), 623-631.

NBS & MLF, (2017). *Annual Agriculture Sample Survey crop and livestock report*. Tanzania: National Beurau of Statistics and Ministry of Livestock and Fisheries. https://www.nbs.go.tz/nbs/takwimu/Agriculture/201617_AASS%20Report%20_Final.pdf

Nespeca, R., Vaillancourt, J., & Morrow, M., (1997). Validation of a poultry biosecurity survey. *Preventive Veterinary Medicine, 31*(1-2), 73-86.

Office International des Epizooties. (2000). Infectious bursal disease. *Review of Science and Technology Office of International Epizootics*, *19*(2), 527-543.

Pauw, K., & Thurlow, J. (2010). Agricultural growth, poverty, and nutrition in Tanzania. International Food Policy Research Institute discussion papers presented at national food conference, Dar es salaam 2010. https://www.ifpri.org/publication/agricultural-growth-poverty-nutrition-tanzania

Radostits, O., Gay, C., Blood, D., & Hinchcliff, K. (2000). V*eterinary Medicine: A textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*. Harcourt Publishers Ltd. London. https://www.elsevier.com/books/veterinary-medicine/radostits/978-0 7020-2777-2

Rautenschlein, S., Kraemer, C., Vanmarcke, J., & Montiel, E. (2005). Protective efficacy of intermediate and intermediate plus infectious bursal disease virus (IBDV) vaccines against very virulent IBDV in commercial broilers. *Avian Diseases, 49*(2), 231-237.

Rodenberg, J., Sharma, J., Belzer, S., Nordgren, R., & Naqi, S. (1994). Flow cytometric analysis of B cell and T cell subpopulations in specific-pathogen-free chickens infected with infectious bursal disease virus. *Avian Diseases, 38*(1), 16-21.

Rosenberger, J., Cloud, S., & Metz, A. (1987). Use of infectious bursal disease virus variant vaccines in broilers and broiler breeders. *Avian Diseases 19*(1): 717–729

Saif, Y. (1991). Immunosuppression induced by infectious bursal disease virus. *Veterinary Immunology and Immunopathology, 30*(1), 45-50.

Sainsbury, D. (2000). *Poultry health and management: Chickens, Turkeys, Ducks, Geese and Quail*: Blackwell Science Ltd., Oxford. https://www.wiley.com/ enus/Poultry+ Health+and+Management%3A+Chickens%2C

Sali, K. (2019). Overview of Methods Used in the Diagnosis of Infectious Bursal Disease. *Veterinary Medicine Open Journal, 4*(1), 9-17.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, 74*(12), 5463-5467.

Sarachai, C., Chansiripornchai, N., & Sasipreeyajan, J. (2010). Efficacy of infectious bursal disease vaccine in broiler chickens receiving different vaccination programs. *The Thai Journal of Veterinary Medicine, 40*(1), 9-14.

Shah, K., & Maghsoudlou, P. (2016). Enzyme-linked immunosorbent assay (ELISA): The Basics. *British Journal of Hospital Medicine, 77*(7), 98-101.

Sharma, J. M., Kim, I., Rautenschlein, S., & Yeh, H., (2000). Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. *Developmental & Comparative Immunology, 24*(2-3), 223-235.

Simba, D., & Msamanga, G. (1994). Use of cold-chain to assess vaccine exposure to adverse temperatures in rural Tanzania. *East African Medical Journal, 71*(7), 445-446.

Silva, M., Desta, S., & Stapleton, J. (1979). Development of the chicken sector in the Tanzania livestock master plan. Tanzania Master Plan Brief 7, October, 2017. pp1-2. Accessed from. https://core.ac.uk/download/pdf/132697821.pdf

Skeeles, J. K., Lukert, P., Fletcher, O., & Leonard, J. (1979). Immunization studies with a cell-culture-adapted infectious bursal disease virus. *Avian Diseases*, *23*(1), 456-465.

Snyder, D. (1990). Changes in the field status of infectious bursal disease virus. *Avian Pathology*, *19*(1), 419-423.

Sudarshan, M., Sundar, M., Girish, N., Narendra, S., & Patel, N. G. (1994). An evaluation of cold chain system for vaccines in Bangalore. *The Indian Journal of Pediatrics*, *61*(2), 173-178.

Swai, E., Kessy, M., Sanka, P., & Mtui, P. (2011). A serological survey for infectious bursal disease virus antibodies in free-range village chickens in northern Tanzania. *Journal of the South African Veterinary Association*, *82*(1), 32-35.

Tanimura, N., & Sharma, J. (1997). Appearance of T cells in the bursa of Fabricius and cecal tonsils during the acute phase of infectious bursal disease virus infection in chickens. *Avian Diseases*, *41*(3), 638-645.

Tanimura, N., Tsukamoto, K., Nakamura, K., Narita, M., & Maeda, M. (1995). Association between pathogenicity of infectious bursal disease virus and viral antigen distribution detected by immunohistochemistry. *Avian Diseases*, *39*(1), 9-20.

Thakker, Y., & Woods, S. (1992). Storage of vaccines in the community: A weak link in the cold chain. The *British Medical Journal*, *304*(6829), 756-758.

Tiscione, N. B. (2018). The Validation of ELISA Screening According to SWGTOX Recommendations. *Journal of Analytical Toxicology*, *42*(3), 33-34.

Wahome, M. W. (2018). *Infectious bursal disease in indigenous village chickens, ducks and turkeys in Embu county, Kenya: status; knowledge, attitudes and practices of value chain actors*. (Doctoral Dissertation at the University of Nairobi). Institutional Repository at University of Nairobi Research Achieve. http://erepository. uonbi.ac.ke/handle/11295/104063?show=full

Wilcox, G., Flower, R., Baxendale, W., & Mackenzie, J. (1983). Serological survey of wild birds in Australia for the prevalence of antibodies to egg drop syndrome 1976 (EDS-76) and infectious Bursal disease viruses. *Avian Pathology, 12*(1), 135-139.

Yamaguchi, T., Iwata, K., Kobayashi, M., Ogawa, M., Fukushi, H., & Hirai, K. (1996). Epitope mapping of capsid proteins VP2 and VP3 of infectious bursal disease virus. *Archives of Virology, 141*(8), 1493-1507.

APPENDICES

Appendix 1: Questionnaire to assess KAPs of poultry farmers on IBD in Dar es Salaam

Socio-demographic information / Personal particulars of the respondent

Respondent's ID No. ……..

Interviewer initials […….]

Residence of respondent District..... Ward.....

1. How old are you now?.....(Age in complete years). Year of birth ……..
2. Sex of respondent 1. Male …… 2. Female ……
3. What is your current marital status?
   1. Single (never married) ☐
   2. Married ☐
   4. Divorced/ separated ☐
   5. Widowed ☐
4. What is the highest level of education you have completed?
   1. None ☐
   2. Primary ☐
   3. Secondary ☐
   4. Certificates level ☐
   5. Diploma level ☐
   6. Degree level ☐
5. Position in the project: 1.Owner ..... 2.Family member.....
   3. Attendant.....4.Others (Specify).....
6. The number of chickens ……
7. What is your current main occupation?
   1. Commercial poultry keeper .....
   2. Employed .....
   3. Others ..... Specify.....
8. For how long have you kept chickens?.....
9. Types of chickens Kept
   1. Broiler

2. Layers

3. Local

Knowledge of commercial poultry keepers on IBD

10. What major diseases of chickens do you encounter on your farm? Mention them in order of importance.

    1.…….

    2.……..

    3.……

11. Have you heard about IBD? (*The researcher should clarify this question*)

    Yes ☐ No ☐

12. If answered **Yes**, in question 10, where did you hear about this disease?

    1. From veterinary doctors/Extension officers ☐

    2. Occurred on my farm ☐

    3. Occurred on my neighbour farm ☐

    4. Others, specify……

13. If answered **Yes**, in question 10, what clinical signs did the chickens presented?

…….

……

14. Do you know how IBD is transmitted?

    Yes ☐ No ☐

15. If answered **Yes**, in question 13, how is it transmitted?

……

…….

16. If answered **Yes**, in question 13, how do you manage the disease?

    Treating them ☐

    Vaccination ☐

    Leave them to die ☐

The attitude of commercial poultry keepers on IBD

17. Do you think your chicken can get IBD if you don't observe bio-security measures?

    Agree ☐ Neutral ☐ Disagree ☐

18. Can IBD be prevented by vaccination?

    Agree ☐ Neutral ☐ Disagree ☐

19. Would you like to get more information about IBD?

Yes ☐ No ☐ Don't care ☐

20. If answered **Yes** in question20, What special information on IBD would you like to know?

Basic knowledge ☐ Prevention methods ☐

Treatment ☐ Other ☐

21. What are the best ways to get this information to you?

From veterinary doctors ☐

Friends/family ☐

Television ☐

Radio ☐

Other ☐

22. Is the cold chain maintenance important for vaccine effectiveness?

1. Yes….. 2. No…. 3. I don't know ……..

23. Presence of disinfectants in water interfere vaccine function?

1. Yes …. 2. No …… 3. I don't know

24. Is IBD a killer disease in chicken?

1. Agree ……. 2. Disagree …. 3. Neutral ……..

Practices of commercial poultry keepers on IBD

25. Have you ever experience any difficulties on chicken keeping example, chicken death?

Yes ☐ No ☐

26. What do you think could be the major cause of chicken death?

Diseases ☐ mention them ……..

No proper reasons ☐ Others ☐ specify…….

27. Do you clean your chicken house and equipment?

1. Yes …….. 2. No …….

28. How often do you clean the chicken house, feeder and drinkers?

……

29. Do you have a foot bath on the entrance of the poultry house?

1. Yes ……. 2. No …….

30. Do you vaccinate your chicken?

Yes ☐ No ☐

31. Vaccination regimen

1. Once ……..…. 2. Twice ……

32. If answered yes in question 26, Mention which diseases do you vaccinate?

.....

.....

33. Who vaccinates your chicken? If the answer is yes in question 26

Myself ☐ Livestock officer ☐

34. Where do you get the vaccine?

Veterinary shop ☐ livestock officer ☐

35. When you buy vaccine, do they provide information on storage and usage of the vaccine?

Yes ☐ No ☐

36. How do you handle the vaccine from the shop up to poultry house?

……..

37. When do you vaccinate after purchasing the vaccines?

Immediately ☐ after some days ☐ Others ……

38. Which water do you use in reconstituting the vaccine?

Tape water ☐ well water ☐ bottle water ☐

39. Have you ever experience the disease after vaccinating the chickens?

Yes ☐ No ☐

40. If yes which vaccine was that?

…….

THANK YOU

Appendix 2: Checklist for Vaccine Storage and Handling

Vaccine Storage and Handling Checklist				
	Name of drug facility:			
SN	**People**			
		Yes	**No**	**Comment**
1	All staff/personnel handling vaccines have been trained in the maintenance of the cold chain.			
	Equipment			
2	The refrigerator is situated away from heat sources			
3	The refrigerator is situated away from direct sunlight			
4	The refrigerator/cold room is operating normally			
5	There are no vaccines stored in the shelves of the door of the refrigerator			
6	A map or guide to the location of vaccine is on the door of the refrigerator/cold chain			
7	The electrical plugs are clearly marked "Refrigerator: Do not switch off"			
	Cold boxes, Vaccine carrier and coolers			
8	There are readily accessible written procedures for packing vaccine into cold boxes, vaccine carriers and/or coolers			
	Monitoring equipment			
9	A separate temperature recording chart/graph/sheet is used for each refrigerator			
10	The current maximum and minimum temperatures have been recorded twice a day			
11	The temperature record is kept close to the refrigerator			
12	The daily temperature records are signed by the person taking the reading			
13	Information about activities such as defrosting, etc., that may affect temperature is recorded			

14	There are procedures describing the action to be taken if temperatures outside the recommended range are recorded			
15	Written vaccine storage and handling procedures are readily accessible to relevant staff			
16	Were there any cold chain breaches?			
17	What actions were taken in response to the cold chain breaches?			
	Alternative Vaccine storage			
18	A written procedure for alternative vaccine storage is readily accessible in the event of power failure or equipment breakdown			
19	Alternative storage (eg Cooler or monitored refrigerator) is available for vaccine storage in the event of equipment failure			
20	Is there a standby generator in case of a power cut off?			
	Information provided to vaccine customers			
21	Do the dispensers provide information on vaccine handling from the shop to the point of use?			
22	Does the Information on how to reconstitute and administer vaccine is provided?			

Appendix 3: Informed Consent Form

THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY

ID number……

Introduction

Greetings! My name is Rukia Saidi, I am a student doing Master's in Global Health and Biomedical Sciences at Nelson Mandela African Institution Science and Technology (NM-AIST). I am conducting a research on; knowledge, attitude and practices of commercial poultry farmers with reference to Infectious Bursal Disease transmission and management in Dar es Salaam region.

Purpose of the study

This study has the purpose of collecting information on knowledge, attitude and practices of commercial poultry farmers with reference to Infectious Bursal Disease transmission and management in Dar es Salaam region. You are being asked to participate in this study because you have particular knowledge and experiences that may be important to the study.

What Participation Involves

If you agree to participate in this study the following will occur:

1. You will sit with a trained interviewer and answer questions about knowledge, attitude and practices of commercial poultry farmers with reference to Infectious Bursal Disease transmission and management. The interviewer will be recording your responses in the questionnaire.

2. No identifying information will be collected from you during this interview, except your age, level of education, marital status and your current occupation.

3. You will be interviewed only once for approximately 35 minutes in a private setting.

Confidentiality

I assure you that all the information collected from you will be kept confidential. Only people working in this research study will have access to the information. We will be compiling a report, which will contain responses from several poultry farmers without any reference to individuals. We will not put your name or other identifying information on the records of the information you provide.

Risks

You will be asked questions about knowledge, attitude and practices of commercial poultry farmers with reference to Infectious Bursal Disease transmission and management. Some questions could potentially make you feel uncomfortable. You may refuse to answer any particular question and may stop the interview at any time.

Rights to Withdraw and Alternatives

Taking part in this study is completely your choice. If you choose not to participate in the study or if you decide to stop participating in the study you will not get any harm. You can stop participating in this study at any time, even if you have already given your consent. Refusal to participate or withdrawal from the study will not affect the quality of service to your chickens that are delivered by livestock officers.

Benefits

There will be no direct benefit to you, however, the information you provide will help to increase our understanding on knowledge, attitude and practices of commercial poultry farmers with reference to Infectious Bursal Disease transmission and management and prepare effective education interventions/programs to the general public on issues related to Infectious Bursal Disease transmission and management. The individual benefit may include advice on good animal husbandry practice that would make you maximize your profit.

In Case of Injury

We do not anticipate that any harm will occur to you or your family as a result of participation in this study.

Who to contact

If you have questions about this study, you should contact Studytudy Coordinator or the **Principal Investigator, Rukia Saidi,** Nelson Mandela African Institution Science and Technology (NM-AIST), P.O. Box 447, Arusha (Tel. no. 0717 210782 or 0767 210782). If you have questions about your rights as a participant, you may call **Dr. Gabriel Mkilema Shirima who is the supervisor** of this study (Tel. no. 0763 973003)

Certification of consent

I have been invited to take part in the study on knowledge, attitude and practices of commercial poultry farmers with reference to Infectious Bursal Disease transmission and management. I have read the foregoing information or it has been read to me and has understood. My questions have been answered to my satisfaction. I agree to participate in this study.

Signature

Do you agree?

Participant Agrees [__]

Participant disagree [__]

Signature (or thumbprint) of participant …….

Signature of witness (if the participant cannot read) …….

Signature of research assistant ……..

Date consent signed ……

Appendix 4: Infectious Bursal Disease Strains used in the Phylogenetic Tree

Virus strain	Accession no	Origin	Genotype
BJ836	AF413069.1	China	Classical
IBD071R	KT633995.1	Iran	Classical
534-North-Carolina	MF142546.1	USA	Classical
IBDV76/NVR1	JX424075.1	Nigeria	Classical
D78	EU162087.1	USA	Classical
03-27950-dn	EF138988.1	Canada	Classical
GBF-1	D16828.1	Japan	Classical
JG011/KEN/15	KY407590.1	Kenya	VV-EU Type
213-048-2	KY556581.1	Brazil	VV-EU Type
713-Russia	MF142562.1	Russia	VV-EU Type
KM523629.1	IBD10HLJ02	China	VV-EU Type
West Bengal/HBL-07-15-b	KT630855.1	India	VV-EU Type
V90/TW95	JQ315162.1	Taiwan	VV-EU Type
IBDV78/ABIC vaccine	IBD10HLJ02	Russia	VV-EU Type
UK661	KT630855.1	China	VV-EU Type
D6948	JQ315162.1	India	VV-EU Type
KMRG-40	AB200982.1	Tanzania	VV-A Type
KARS-53	AB200984.1	Tanzania	VV-A Type
KMZA-78	AB200985.1	Tanzania	VV-A Type
LUSC47-2016	LC319700.1	Zambia	VV-A Type

Appendix 5: KNCHREC Ethical Clearance

Kibong'oto Infectious Diseases Hospital- Nelson Mandela African Institution of Science and Technology- Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) -KNCHREC

RESEARCH ETHICAL CLEARANCE CERTIFICATE

Research Proposal No: KNCHREC0013 **14TH MARCH 2019**

Study Title: PERFORMANCE OF COMMERCIAL VACCINES AGAINST LOCALLY ISOLATED INFECTIOUS BURSAL DISEASE VIRUSES IN CHICKEN IN TANZANIA

Study Area: THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY

PI Name: Rukia Saidi
Co-Invigilator:
Institutions: School of Life Science and Bio-Engineering (LiSBE) of the Nelson Mandela African Institution of Science and Technology

The Proposal has been approved by KNCHREC on 14th March 2019

1. Subject to this approval you will be required to submit your progress report to the KNCHREC, National Institute of Research and Ministry of Health Community Development Gender Elderly and Children
2. Publication of your findings is subject to presentation to the KNCREC and NIMR Approval.
3. Copies of final publication should be made available to KNCHREC, National Institute of Research and Director of Veterinary Services

Duration of Study Renewal: Subject to Renewal within ONE YEAR
Span From: 14th March 2019 to 13TH March 2020.

.....
Mr. Simon Njeya
Secretary
KNCHREC

Chairperson
KNCHREC